Vol. 49 April 1960 No. 4

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

CONTENTS OF THIS ISSUE

An Evaluation of Certain Hypotensive Agents III. Tetrahydroisoquinoline and Tetrahydroquinoline Derivatives 183
R. K. Bickerton, M. L. Jacquart, W. J. Kinnard, Jr., J. A. Bianculli, and *J. P. Buckley*

The Alkaloids of *Argemone munita* subsp. *rotundata* 187
Lemont B. Kier and *Taito O. Soine*

Preparation and Properties of New Gastric Antacids I. Aluminum Hydroxide-Magnesium Carbonate Dried Gels 191
Stewart M. Beekman

Preparation and Properties of New Gastric Antacids II. Aluminum Hydroxide-Protein Dried Gels 201
Stewart M. Beekman and *Carl H. Vogel*

The Effects of Certain Psychopharmacologic Drugs on Conditioning in the Rat I. Avoidance-Escape Conditioning 205
V. D. Lynch, M. D. Aceto, and *R. K. Thoms*

The Antidotal Effectiveness of Sodium Cobaltinitrite in Antagonizing Cyanide Poisoning in Albino Mice 210
Marvin M. Goldenberg and *David E. Mann, Jr.*

A Comprehensive Pharmaceutical Stability Testing Laboratory III. A Light Stability Cabinet for Evaluating the Photosensitivity of Pharmaceuticals 213
Leon Lachman, Charles J. Swartz, and *Jack Cooper*

A Preliminary Pharmacologic Investigation of the Roots of *Bixa orellana* 218
Norris W. Dunham and *Kenton R. Allard*

An Investigation of Some Pharmaceutical Applications of Certain Fatty Acid Esters of Sucrose 220
Howard Hopkins and *LaVerne D. Small*

The Effects of Tranquilizers on Bacterial Toxemias I. Reserpine 225
Leo Greenberg, James W. Ingalls, and *Arthur G. Zupko*

Binding of Drugs by Plastics II. Interaction of Weak Organic Acids with Plastic Syringes 227
H. K. Kim and *J. Autian*

Studies on the Inhibitory Effect of Combined Chemical Preservatives on *Saccharomyces cerevisiae* 231
H. G. Osman and *Afaf El-Mariah*

Influence of Hydrophil-Lipophil Balance on Ointment Bases 234
John W. Rhyne, William J. Payne, and *Charles W. Hartman*

The Interaction of Citrate With Aspirin and Benzoic Acid 237
Sanford Bolton

Veratrum Alkaloids XLIII. The Structure of Cevadine 242
S. Morris Kupchan and *Adriano Afonso*

Some Pharmaceutical Properties of Novobiocin 245
John D. Mullins and *Thomas J. Macek*

The Effect of Thixotropy on Suspension Stability 249
Ernest C. Foernzler, Alfred N. Martin, and *Gilbert S. Banker*

A Note on the Photolytic Degradation of Anti-inflammatory Steroids 253
W. E. Hamlin, T. Chulski, R. H. Johnson, and *J. G. Wagner*

A Note on an Infrared Assay of Chloroform in Pharmaceutical Products 255
John C. Souder and *Patrick Deluca*

A Note on the Occurrence of 2,6-Dimethoxybenzoquinone in *Rauwolfia vomitoria* 257
S. Morris Kupchan and *Mang E. Obasi*

Book Notices 258

Advertising ..... Cover pages 2, 3, and 4, and I to IV

© *Copyright 1960 by the American Pharmaceutical Association*

MEMO TO:
White Oil and Petrolatum Users
PENN-DRAKE TECHNICAL SERVICE always is at YOUR SERVICE
. . . to help you make a good product better—or to provide valued assistance in the solution of your problems. May we consult with you on any White Oil or Petrolatum application?

penn drake products

PENNSYLVANIA REFINING COMPANY

BUTLER 32, PENNSYLVANIA

BRANCHES: CLEVELAND, OHIO AND EDGEWATER, N. J.

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

SCIENTIFIC EDITION

Committee on Publications

Robert P. Fischelis, *Chairman*
William S. Apple
Grover C. Bowles
J. Warren Lansdowne
Hugo H. Schaefer

Editorial Advisory Board

J. H. Burckhalter
Troy C. Daniels
George P. Hager
W. W. Hilty
Thomas J. Macek
Varro E. Tyler, Jr.

JUSTIN L. POWERS, EDITOR
EDWARD G. FELDMANN, ASSOCIATE EDITOR
SAMUEL W. GOLDSTEIN, ASSISTANT EDITOR
YVETTE W. GORDON, EDITORIAL ASSISTANT

The Massachusetts College of Pharmacy

Longwood Avenue, Boston

Graduate study in Boston has always appealed strongly to students in the health professions. The environment is stimulating and attracts students from all parts of the world. Library, laboratory, hospital, and other facilities for graduate work are outstanding in this area.

The Massachusetts College of Pharmacy is located in this great educational center. It offers unusual opportunities to graduates of colleges of pharmacy who are interested in further study in pharmacy, pharmaceutical chemistry, and pharmacognosy.

For additional information, write to HOWARD C. NEWTON, Dean

The *Journal of the American Pharmaceutical Association* is published semimonthly by the American Pharmaceutical Association as a *Scientific Edition* and a *Practical Pharmacy Edition*.

Offices: Editorial, Advertising and Subscription Offices: 2215 Constitution Avenue, N.W., Washington 7, D.C. Publication Office: 20th and Northampton Streets, Easton, Pennsylvania.

Annual Subscription—United States and foreign, *Practical Pharmacy Edition* $10.00, *Scientific Edition* $15.00, both editions $20.00. Subscription rates are subject to change without notice. Members of the American Pharmaceutical Association receive the *Practical Pharmacy Edition* each month as a part of their annual A.Ph.A. membership dues, and may subscribe to the *Scientific Edition* at the special rate of $2.00. Single copies—*Practical Pharmacy Edition*, $1.00; *Scientific Edition*, $1.50.

Claims—Missing numbers will not be supplied if claims are received more than 60 days after the date of the issue, or if loss was due to failure to give notice of change of address. The Association cannot accept responsibility for foreign delivery when its records indicate shipment has been made.

Change of Address—Members and subscribers should notify at once both the Post Office and the American Pharmaceutical Association, 2215 Constitution Avenue, N.W., Washington 7, D.C. of any change of address. Second Class Postage paid at Easton, Pa., and additional mailing offices. Issued 24 times a year; *Scientific Edition* monthly on the 20th and *Practical Pharmacy Edition* monthly on the 10th.

© Copyright 1960, American Pharmaceutical Association, 2215 Constitution Ave., N.W., Washington 7, D.C.

PHARMACEUTICAL CHEMIST

Opportunity in Merck Product Development

Excellent position for pharmacist or pharmaceutical chemist. Will perform independent studies of pharmaceutical formulations and techniques. Work with new or existing medicinals and therapeutic agents to develop new products. Experience in product development and B.S. degree in pharmacy or pharmaceutical chemistry required. Graduate degree preferred.

MERCK

Please send resume to

G. A. DUCCA
Merck & Co., Inc.
Rahway, New Jersey

NOW—

An attractive PERMANENT BINDER for the Journal of the American Pharmaceutical Association

- ★ Do your own binding in only a matter of minutes
- ★ Opens flat as a bound book
- ★ Made of durable, imitation leather
- ★ Will preserve your Journals permanently
- ★ Each cover holds 12 issues
- ★ Easy to follow instructions
- ★ Stamped with title, year and volume number

Combination sets:	*Single Editions:*
(To 1957) **$5.50** pr.	Scientific **$3.25** ea.
(1957 on) **$6.00** pr.	Practical (Old size) **$3.25** ea.
	Practical (New size) **$3.75** ea

Add 30¢ per binder for postage and handling in U.S.—Foreign postage extra.

SUCKERT LOOSE LEAF COVER CO.
11911 Grand River Ave., Detroit 4, Michigan

Botanicals

by Meer offer you

Reliability
Stability
Standardization
Uniformity

MAKING YOUR PRODUCTS MORE ATTRACTIVE TO YOUR CUSTOMERS...GET THE MEER CATALOG AND PRICE LIST BY WRITING TODAY!

Meer Corporation

Warehoused at:
318 West 40th Street, New York 36, N. Y. • JUdson 6-0900
325 West Huron Street, Chicago 10, Ill. • Michigan 2-8895
Plant: 9412 Railroad Avenue, North Bergen, New Jersey
Canada: P. N. Sodex & Co., Ltd., Toronto 18 • Montreal 29

♦ COMPLETE ♦
LABORATORY SERVICE

Animal Toxicity Studies. Human Patch Tests. Radioactive Tests. INFRARED SPECTROSCOPY. Pilot plant for product development. Bacteriological testing and studies. Let the most versatile and best equipped laboratory in the midwest solve your problems. *Brochure on request.*

SCIENTIFIC ASSOCIATES
3755 Forest Park Ave.
St. Louis 8, Mo.—JE. 1-5922

NOTICE TO AUTHORS

Reprint copies of *Notice to Authors* are available upon request from the editorial office. The *Notice* details information for the preparation of manuscripts to be considered for publication in THIS JOURNAL. Single copies of the reprint will be sent free of charge. Requests should be directed to:

Scientific Edition
Journal of the American
Pharmaceutical Association
2215 Constitution Avenue, N. W.
Washington 7, D. C.

PHARMACEUTICAL RESEARCH

Expansion of our facilities has created several responsible opportunities in the research and development of ethical pharmaceuticals.

SENIOR PHARMACOLOGIST—For the pharmacologic study of CNS acting agents. Background should include a Ph.D. in pharmacology with some industrial experience.

PHARMACEUTICAL CHEMIST—Pharmaceutical research and product development. Background should include a Ph.D. in pharmaceutical chemistry or pharmacy with 1–3 years' industrial experience.

Laboratory facilities and employee benefit programs are excellent. Location is in a pleasant suburb northwest of Chicago.

Please reply in confidence stating personal data, education, experience and salary history.

BAXTER LABORATORIES, INC.
Morton Grove, Illinois

LABORATORY SERVICES for the FOOD & DRUG INDUSTRIES

Drug Evaluation, Food Additive Studies,
Chemical and Biological Assays,
Clinical Studies, Research

LAWALL & HARRISSON
Div. P 1921 Walnut St., Philadelphia 3, Pa. LO 3-4322

BACK ISSUES

Back issues of almost all volumes of the *Journal of the American Pharmaceutical Association, Scientific Edition*, are available on order from the Association. Orders or inquiries regarding the availability of specific issues and price quotations should clearly indicate "SCIENTIFIC EDITION," and specify the number of copies desired, the month and year of each issue, and preferably the volume number.

Address correspondence to:

Order Department
American Pharmaceutical Association
2215 Constitution Avenue, N. W.
Washington 7, D. C.

New Edition Now Available

THE MERCK INDEX

Now Greatly Expanded in Size and Scope

The all-new MERCK INDEX—outdating previous editions—is now ready for your reference. Published on a nonprofit basis to serve the professions, this new 7th edition is completely updated, increased in size, and expanded by 2,300 chemical monographs. New cross-indexing quickly leads you to concise information on any given substance—regardless of whether you look it up under brand name, generic name or chemical name. Send coupon now to receive your copy.

Luxuriously bound **Contains 1600 pages** **$12.00**

MAIL TODAY

Merck & Co, Inc.
Rahway, New Jersey **Dept. J. S.4**

Please send me a copy of the new MERCK INDEX (Seventh Edition).

. . . . I enclose $12.00 to save handling charge

. . . . Bill me for $12.00 plus 40¢ handling charge

Name . . . . . .

Address . . . . . .

City . . . . . Zone . . . State . . . . . .

AMERICAN CHOLESTEROL PRODUCTS, INC.
build better products with . . .
MODULAN
Acetylated Lanolin
Modulan is an extraordinarily protective and soothing emollient for use on the most delicate tissues. It imparts soft waxy hydrophobic films to skin and hair in both emulsified and anhydrous preparations such as ointments, lotions, solutions, soaps and shampoos. Modulan is almost odorless, oil soluble and hypo-allergenic.
AMERCHOL
Cholesterol Ointment Bases and Emulsifiers
are natural (lanolin-derived) hydrophilic products which form stable pharmaceutical ointment vehicles. The Amerchols induce rapid drug release and promote optimum healing rates. They are non-irritating and hypo-allergenic, possessing unusual emollient and spreading properties.
American Cholesterol Products INCORPORATED
AMERCHOL PARK • EDISON, NEW JERSEY

NOTICE

PAPERS FOR SECTION MEETINGS

OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

The next annual meeting of the American Pharmaceutical Association will be held in Washington, D. C., August 14 to 19, 1960. Titles and abstracts (not to exceed 200 words) of papers must be submitted to the appropriate Section secretary not later than May 15, 1960, or preferably earlier, in order to assure a place on any Section program.

SECTION SECRETARIES

SCIENTIFIC
ROBERT C. ANDERSON
Eli Lilly and Co.
Indianapolis, Ind.

EDUCATION AND LEGISLATION
CECIL P. HEADLEE
C. B. Kendall Co.
2039 Madison Ave.
Indianapolis, Ind.

HISTORICAL PHARMACY
DONALD T. MEREDITH
The Upjohn Co.
Kalamazoo, Mich.

MILITARY PHARMACY
CMDR. CARL BROWN
4310 Chestnut St.
Bethesda 14, Md.

PRACTICAL PHARMACY
SAMUEL W. GOLDSTEIN
2215 Constitution Ave., N.W.
Washington, D. C.

PHARMACEUTICAL ECONOMICS
BENJAMIN A. SMITH
Eli Lilly and Co.
Indianapolis, Ind.

PHARMACY STUDENTS
ROSE MARIE WILKAS
3638 So. Lowe Ave.
Chicago 9, Ill.

INDUSTRIAL PHARMACY
EARL KIMES
Smith Kline & French
1500 Spring Garden St.
Philadelphia, Pa.

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME 49 | *APRIL 1960* | NUMBER 4

An Evaluation of Certain Hypotensive Agents III*

Tetrahydroisoquinoline and Tetrahydroquinoline Derivatives

By R. K. BICKERTON†, M. L. JACQUART, W. J. KINNARD, Jr., J. A. BIANCULLI, and J. P. BUCKLEY

The hypotensive activity of a series of diquaternarized tetrahydroisoquinoline and tetrahydroquinoline derivatives was investigated in the anesthetized rat. The compounds were evaluated according to the mean duration of action of the dose of the compound producing a mean drop in blood pressure of approximately 50 per cent. Several of the compounds were further evaluated in normotensive dogs. The most active compounds were 1-(1,2,3,4-tetrahydroisoquinolino)-5-dimethylamino-3-hydroxy-3-ethinyl pentane dimethobromide (JB-5037), N-(3-dimethylaminopropyl)-1,2,3,4-tetrahydroisoquinoline dimethobromide (JB-621, IN-243) and 1-(1,2,3,4-tetrahydroisoquinolino)-5-dimethylamino-2-pentyne dimethiodide (JB-679).

BURN AND DALE (1) first described the interruption of ganglionic transmission by tetraethylammonium ion in 1914. Acheson, Moe, and Pereira (2, 3) later described the relatively pure ganglionic blocking properties of tetraethylammonium bromide. Since this time both quaternary and bis-quaternary ammonium compounds have been extensively investigated in search of a compound suitable for use in the treatment of hypertensive vascular disease. O'Dell, Luna, and Napoli (4) reported the hypotensive activity of some bis-quaternary ammonium compounds and found that N-(3-dimethylaminopropyl) - 1, 2, 3, 4 - tetrahydroisoquinoline dimethobromide possessed the most potent hypotensive activity of a series of tetrahydroisoquinoline derivatives when investigated in dogs. Biel and Di Pierro (5) had previously reported the synthesis and relative hypotensive activity of some quaternary salts of N-(4-amino-2 - butynyl) - 1, 2, 3, 4 - tetrahydroisoquinoline derivatives. This present report deals principally with the evaluation of the hypotensive activity in rats and dogs of certain diquaternarized tetrahydroisoquinoline and tetrahydroquinoline derivatives (Fig. 1) which have been synthesized by Biel and his co-workers.

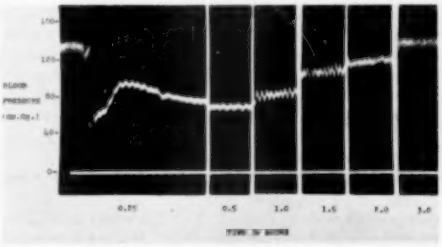


Fig. 1.—The effects of 2.5 mg./Kg. of JB-621 on the blood pressure of a normotensive dog.

EXPERIMENTAL

Hypotensive Activity in Normotensive Rats.—The compounds were screened and evaluated for their hypotensive activity in anesthetized normotensive rats using a modification of a method described in a previous communication from this lab-

* Received August 21, 1959, from the University of Pittsburgh, School of Pharmacy, Pittsburgh, Pa.

† George A. Kelly, Sr., Fellow.

This investigation was supported in part by a research grant from Lakeside Laboratories, Milwaukee, Wis.

The Experimental compounds were kindly supplied by Dr. H. L. Daiell, Scientific Director, Lakeside Laboratories, Inc.

Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.

oratory (6). Albino Wistar rats weighing approximately 150 Gm. were anesthetized by an intraperitoneal injection of 1.2 Gm./Kg. of urethan and secured to an animal operating board. The left common carotid artery was cannulated. Normal saline solution was used in the system in place of the 7.5% sodium citrate solution. A minimum of thirty minutes was allowed to elapse prior to drug administration to enable the blood pressure to stabilize. All the compounds were dissolved, just before use, in distilled water prior to being administered into a femoral vein. The initial dose of each compound was 1 mg./Kg., and subsequent dosage was adjusted according to the results obtained in order to determine the dose of a particular compound that would produce a drop in blood pressure of approximately 50%. Dose-responses were plotted on semilog paper and the dose producing a 50% drop in blood pressure was estimated. In some instances it was necessary to utilize a dose of a compound which induced a milder depressor effect because of the obvious signs of toxicity or occasional lethal effect at the higher dose level. The approximate ED_{50} was then administered to a minimum of four rats (two of each sex), and the mean per cent drop in arterial pressure and the mean time for the blood pressure to return to predrug levels determined. The number of animals used in the evaluation of each compound varied according to the reproducibility of results. Chlorisondamine and pentolinium were used as the control compounds.

Hypotensive Activity in Normotensive Dogs.—Certain of the compounds were further evaluated in normotensive dogs to study the possibility of species variation. Mongrel dogs of both sexes were anesthetized with pentobarbital sodium, 35 mg./Kg., I. V. Direct blood pressure recordings were obtained from either the right femoral artery or one of the carotid arteries. Freshly prepared solutions of the compounds were administered via one of the femoral veins.

RESULTS

Hypotensive Activities in Normotensive Rats.—The hypotensive activity of the experimental compounds in rats is summarized in Table I. The compounds were evaluated on the basis of the duration of action of the respective effective doses and not on a dose-dose relationship. The rating used was as follows: +, (relatively poor hypotensive activity), 0–40 minutes duration; ++, (good hypotensive activity), 41–80 minutes; +++, (very good hypotensive activity), 81–120 minutes; ++++, (excellent hypotensive activity), 121+ minutes.

All the experimental compounds showed some degree of hypotensive activity. The most active compounds were JB-5037, 1-(1,2,3,4-tetrahydroisoquinolino)-5-dimethylamino-3-hydroxy-3-ethinyl pentane dimethobromide, JB-621, N-(3-dimethylaminopropyl)-1,2,3,4-tetrahydroisoquinoline dimethobromide, and JB-679, 1-(1,2,3,4-tetrahydroisoquinolino)-5-dimethylamino-2-pentyne dimethiodide. The remaining experimental compounds were relatively mild hypotensive agents in comparison to the control compounds investigated.

Hypotensive Activity in Normotensive Dogs.—Compounds JB-604, JB-605, JB-610, JB-611, JB-621, JB-633, JB-637, JB-638, JB-679, and JB-5037 were further investigated in normotensive dogs. The results are summarized in Table II. None of the compounds was as active as the esters of piperidine carboxylic acid previously reported by this laboratory (6). Intravenous administration of 0.75 mg./Kg. and 2.5 mg./Kg. of JB-621 to a dog produced a biphasic hypotensive response comparable to that reported by O'Dell (Fig. 1).

All of the compounds produced biphasic drops in pressure with the secondary depressor effect occurring more slowly and usually to a somewhat lesser extent than the initial fall in blood pressure.

DISCUSSION

In group one, see Fig. 2, the butynyl derivatives of a series of diquaternarized tetrahydroisoquinolines were relatively weak hypotensive agents. However the saturated propyl derivative N-(3-dimethylaminopropyl)-1,2,3,4-tetrahydroisoquinoline dimethobromide (JB-621, IN-243 Irwin, Neisler and Co.) was very active, having a mean duration of action of one hundred and two minutes. The tetrahydroisoquinoline derivative containing an acetylenic branch in the side chain (JB-5037), appeared to be one of the most active compounds investigated. Within group one, acetylenic derivatives, replacement of the terminal alkyl substituted ammonium nitrogen (JB-610) with a heterocyclic structure such as piperidino (JB-620) or pyrrolidino (JB-618) increased the depressor effect but decreased the duration of action of equal doses. Increasing chain length from four carbons (JB-610) to five carbons (JB-679) increased the hypotensive activity four times. It should be noted here that 7.5 mg./Kg. of JB-679 was selected as the rat-evaluating dose although 10 mg./Kg. more closely produced the 50 per cent drops in mean pressure. However four out of six animals died at this dose level (10 mg./Kg.).

In group two, see Fig. 3, similar structure-activity relationships were observed. Replacement of ter-

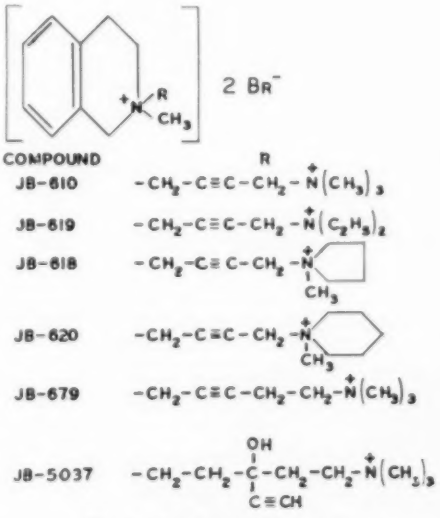


Fig. 2.—Structures of group one.

TABLE I.—THE EVALUATION OF TETRAHYDROISOQUINOLINE AND TETRAHYDROQUINOLINE DERIVATIVES ON THE BLOOD PRESSURE OF NORMOTENSIVE RATS

Compound JB-	Dose, mg./Kg.	No. of Animals	Mean % Drop Min. ± S. D.	Mean Time to Return to Predrug Levels Min. ± S. D.	Activity
610	20.0	4	62.6 ± 22.3	52.0 ± 35.7	++
618	5.0	4	44.3 ± 8.7	17.2 ± 10.5	+
619	5.0	4	49.9 ± 12.1	13.0 ± 6.7	+
620	5.0	5	40.4 ± 10.6	5.1 ± 2.3	+
679	7.5	6	34.7 ± 7.1	210 5 ± 110.1	++++
5037	5.0	12	43.7 ± 9.27	139.4 ± 65.9	++++
604	20.0	4	47.4 ± 19.1	40.6 ± 24.0	++
605	20.0	4	48.4 ± 6.2	63.0 ± 2.1	++
636	10.0	5	51.5 ± 11.9	7.0 ± 3.8	+
637	10 0	5	50.4 ± 3.5	70.2 ± 24.5	++
638	25 0	4	48.5 ± 1.9	54.5 ± 21.7	++
617	5.0	4	42.3 ± 8.8	27.5 ± 9.9	+
611	5.0	4	38.4 ± 5.9	13.1 ± 9.7	+
629	10.0	4	48.5 ± 8.2	45.25 ± 13.3	++
633	10.0	8	44.9 ± 5.6	10.6 ± 7.1	+
621	5.0	10	57.08 ± 15.85	102.66 ± 46.6	+++
655	10.0	4	38.2 ± 4.4	3.9 ± 1.2	+
Pentolinium	1.0	11	48.7 ± 18.6	118.5 ± 40.3	+++
Chlorisondamine	0.5	14	44.6 ± 13.8	141.2 ± 51.6	++++

TABLE II.—THE EFFECTS OF CERTAIN HYPOTENSIVE AGENTS ON THE BLOOD PRESSURE OF NORMOTENSIVE DOGS

Compound JB-	Dose, mg./Kg.	Mean Drop in Blood Pressure, %	Time to Return to Original Levels, min.
604	10	71.6	78
605	10	79.4	113
605	20	49.2	390
610	10	47.3	758+
610	5	54.0	207
633	5	31.4	28
633	30	71.7	198 (died)
637	5	37.9	169
637	10	39.0	121
638	10	50.6	188
638	15	36.8	206
621	5	46.0	327
621	2.5	48.0	170
621	0.75	21.0	80
5037	10	28.0	463
5037	5	27.0	670
5037	2.5	24.0	420
5037	2.5	46.0	247

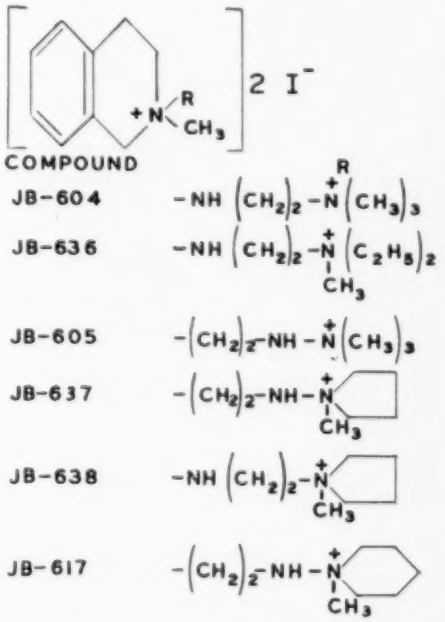
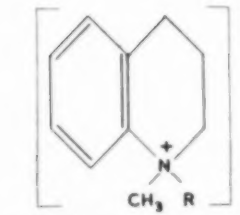


Fig. 3.—Structures of group two.

minal methyl groups (JB-604) with ethyl groups (JB-636) increased the hypotensive depressor effect but decreased the duration of action from respective doses. The position of the extra nitrogen forming a hydrazine analog appeared critical only when heterocyclic ammonium structures were the terminal group. In this case increased activity was observed when one of the hydrazine nitrogens was linked to the terminal ammonium nitrogen (JB-637) rather than the tetrahydroisoquinoline nitrogen (JB-638). In group three (Fig. 4), tetrahydroquinoline derivatives, increased duration of hypotensive activity resulted when the alkyl substituted terminal ammonium nitrogen was replaced with a pyrrolidino radical. Depressor responses to equal doses remained unchanged.

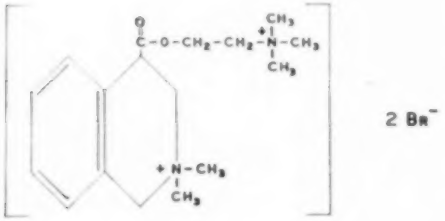
Among the compounds in groups two and three, JB-604, the tetrahydroisoquinoline isomer of JB-611, required a larger dose to produce a 50% drop in blood pressure but at the same time induced a longer duration of action. JB-633 and JB-636, also structural isomers, were qualitatively the same. JB-629 and JB-638, the remaining structural isomers, appeared similar in activity excepting that JB-629 was more active on a dose–dose basis.

JB-655 (Fig. 5), the only tetrahydroisoquinoline carboxylate dimethobromide of the series was also the least active compound. JB-621 (IN-243) (Fig. 6) which contains a saturated side chain and an alkyl substituted terminal ammonium nitrogen was one of the most active compounds investigated.



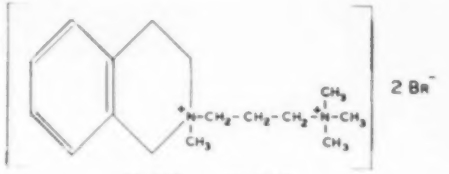
COMPOUND	R
JB-611	$-NH-CH_2-CH_2-\overset{+}{N}(CH_3)_3$
JB-633	$-NH-CH_2-CH_2-\overset{+}{N}(C_2H_5)_2CH_3$
JB-629	$-NH-CH_2-CH_2-\overset{+}{N}$ (pyrrolidinium) CH_3

Fig. 4.—Structures of group three.



JB-655

Fig. 5.—Structure of Compound JB-655.



JB-621 IN-243

Fig. 6.—Structure of Compound JB-621.

SUMMARY

1. The hypotensive activity of a series of tetrahydroisoquinoline and tetrahydroquinoline derivatives was investigated and evaluated.

2. The 2-butynyl derivatives of tetrahydroisoquinoline were relatively weak hypotensive agents as were the diquaternarized hydrazine analogs.

3. JB - 679, 1 - (1,2,3,4 - tetrahydroisoquinolino) - 5 - dimethylamino - 2 - pentyne dimethiodide, JB - 5037, 1 - (1,2,3,4 - tetrahydroisoquinolino) - 5 - dimethylamino - 3 - hydroxy-3-ethinyl pentane dimethobromide, and JB - 621, N - (3 - dimethylamino - propyl)-1,2,3,4 - tetrahydroisoquinoline dimethobromide were the most active compounds in the current series.

REFERENCES

(1) Burn, J. H., and Dale, H. H., *J. Pharmacol. Expil. Therap.*, **5**, 417(1914).
(2) Acheson, G. H., and Pereira, S. A. *ibid.*, **87**, 273 (1946).
(3) Acheson, G. H., and Moe, G. K., *ibid.*, **87**, 220(1946).
(4) O'Dell, T. B., Luna, C., and Napoli, M. D., *ibid.*, **114**, 317(1955).
(5) Biel, J. H., and Di Pierro, F., *J. Am. Chem. Soc.*, **80**, 4609(1958).
(6) Hudak, W. J., Buckley, J. P., Schalit, F. M., and Reif, E. C., THIS JOURNAL, **46**, 595(1957).

The Alkaloids of *Argemone munita* subsp. *rotundata**

By LEMONT B. KIER† and TAITO O. SOINE

Continuing studies in these laboratories with respect to the alkaloidal content of Argemone species have led the authors to examine *Argemone munita* subsp. *rotundata* obtained from the vicinity of Nephi, Utah. The usual isolation techniques have led to a crystalline base (m. p. 245–245.5°), provisionally named rotundine, as the principal alkaloid in the overground portions of the plant. Other alkaloids in the plant material are present in minute amounts and have not been obtained in sufficient quantity for detailed study. Analysis of rotundine established the formula, $C_{19}H_{21}NO_4$, which has been expanded further by functional group analysis to $C_{16}H_{10}(OCH_3)_2(OH)_2(NCH_3)$. Methylation of the phenolic base with diazomethane yielded a base, $C_{21}H_{25}NO_4$, identical with argemonine, a base previously isolated from *Argemone hispida*. Pharmacologic examination of the new base revealed a slight to moderate inhibition of motor activity as the principal action combined with a low toxicity.

ARGEMONE MUNITA Dur. and Hilg. subsp. *rotundata* (Rydb.) G. B. Ownb. is a perennial found growing along roads, washes, and denuded land and is regarded as a pioneer species. It has been known by a number of other names including *A. rotundata* Rybd., *A. hispida* Torrey, and *A. Mexicana* Torrey. Henceforth in this paper, the plant will be referred to as *A. munita* subsp. *rotundata*. It is found in Utah, Nevada, and southern California. The plant itself has a stout stem which is densely and strongly bristled. The cauline leaves are bristly, especially on the veins and margins and they are usually round-lobed. The calyx also is bristly with erect or slightly spreading horns. The petals are white and fully 4–6 cm. long. The pod is ovate to narrowly elliptical and very bristly.

A search of the literature reveals that *A. munita* subsp. *rotundata* has not been examined for its alkaloidal content under any of its botanical names. A few other species have been studied, namely *A. Mexicana* (1–6), *A. alba* (7), *A platyceras* (8) and, *A. hispida* G. (9, 10). The first three species named have contained alkaloids, principally of the cryptopine, berberine, and sanguinarine types. The bases in *A. hispida* G., however on the basis of a preliminary study (10), do not appear to conform to these types. According to Ownbey (11), it is definitely possible that *A. munita* subsp. *rotundata* is the same species that was orginally investigated by Soine and Gisvold (9) as *A. hispida* G. The true species, *A. hispida* G., is said to be found in a narrow belt running down the eastern side of the Rocky Mountains from southeastern Montana, through Colorado, to northern Arizona whereas the species investigated by Soine and Gisvold was collected in the vicinity of Reno, Nevada, where only *A. munita* subsp. *rotundata* is found. It would appear, therefore, that it was impossible for *A. hispida* G. to have been the subject of their investigation and that they actually studied *A. munita* subsp. *rotundata*. Unfortunately, for lack of sufficient material, the authors were unable to study the Nevada-grown *A. munita* subsp. *rotundata* to prove or disprove this fact. Specimens of the Nevada plant, however, have been compared with Utah-grown *A. munita* subsp. *rotundata* and found to be morphologically identical. Inasmuch as a plentiful supply of the Utah-grown plant was available, it was chosen as the material for further study.

The results of this investigation have indicated a difference in the alkaloids found as against those previously isolated from *A. hispida* G. The alkaloids found earlier were nonphenolic argemonine ($C_{21}H_{25}NO_4$) and mono-phenolic *norargemonine* ($C_{20}H_{23}NO_4$), the latter being convertible to the former with diazomethane. The principal new alkaloid, provisionally named *rotundine* ($C_{19}H_{21}NO_4$), isolated in the present study, was diphenolic and, while different from the preceding, also was closely related because it could be methylated to yield argemonine. Therefore, one can assume with assurance that the basic alkaloidal nucleus is the same and it is probable that the originally studied *A. hispida* G. was, indeed *A. munita* subsp. *rotundata* with the alkaloidal differences being accounted for by soil and climatic differences.[1]

Isolation of the alkaloids from *A. munita*

* Received August 21, 1959, from the College of Pharmacy, University of Minnesota, Minneapolis.

† Samuel W. Melendy Fellow, 1957. Present address: College of Pharmacy, University of Florida, Gainesville.

The authors are indebted to Prof. Gerald B. Ownbey, Department of Botany, University of Minnesota, for his advice, counsel, and professional identification of all plant materials. Because he has recently reclassified the entire *Argemone* genus (11) and is probably the country's leading expert on the genus, the authors felt extremely fortunate in being able to obtain his services.

Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.

[1] Batra and Soine (12) have recently examined a sample of true *A. hispida* G. collected in Colorado and have been able to isolate both argemonine and norargemonine as well as to note the presence of other unidentified alkaloids.

subsp. *rotundata* was carried out by the usual method of extracting the dried and powdered over-ground portions of the plant material with alcohol,[2] followed by removal of the solvent and transfer of the alkaloidal bases to a dilute hydrochloric acid solution. This solution of alkaloidal hydrochlorides was then alkalinized with sodium carbonate and extracted with an immiscible solvent to obtain a crude total alkaloidal fraction. These were placed into an acidic solution again and separated into phenolic and nonphenolic fractions by making use of sodium hydroxide as the alkalinizing agent to hold the phenolic bases in the aqueous phase as phenolates while the nonphenolic bases were extracted with an immiscible solvent. The phenolic bases were finally obtained from the alkaline solution by carbonation or by reacidification and sodium carbonate alkalinization followed by immiscible solvent extraction. Attempted purification of the nonphenolic fraction by recrystallization and chromatography indicated the presence of at least two components but the amounts were so small that further work on this fraction was discontinued. Insofar as we were able to determine, argemonine did not appear to be present in the nonphenolic fraction. The phenolic fraction appeared to represent the bulk of the alkaloids present in the plant. This fraction was repeatedly recrystallized and appeared to be composed of a single entity, provisionally named *rotundine*. The homogeneity of *rotundine* was established by paper chromatography. Elemental analysis indicated the presence of carbon, hydrogen, nitrogen, and oxygen. Functional group analysis indicated the presence of two methoxyl groups, one N-methyl group, and two active hydrogens. The presence of two active hydrogens combined with the phenolic character of the base suggested two phenolic groups. From the elemental analysis and molecular weight determination, the molecular formula, $C_{19}H_{21}NO_4$, was assigned. This was extended to $C_{18}H_{19}(OCH_3)_2(OH)_2(NCH_3)$ by the functional group analysis. Optical rotation studies showed that rotundine was strongly levorotatory. Infrared spectra showed strong absorption in the hydroxyl region, confirming the phenolic character, but no absorption was observed in the carbonyl region (see Fig. 1). Methylation of rotundine with diazomethane produced a dimethyl ether with alkaloidal character analyzing for the formula, $C_{21}H_{25}NO_4$. This dimethyl ether was found to be identical with argemonine, previously isolated from *A. hispida* G. (9, 10), by comparison of derivatives as well as by comparison of the infrared spectra of the methiodides (see Fig. 2). Soine and Schermerhorn (10) had previously reported that norargemonine, when methylated, gave argemonine and, therefore, the relationship between these three alkaloids was established. Confirmation of this was found in the similar ultraviolet spectra of the three compounds (see Fig. 3), all apparently containing the same chromophore. A future communication will deal with structural studies that have been carried out on the three alkaloids.

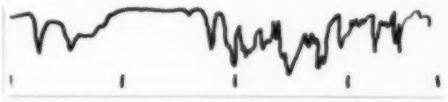


Fig. 1.—Rotundine; phase, potassium bromide.

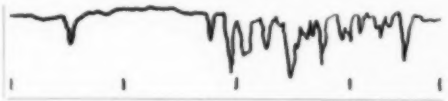


Fig. 2.—Dimethylrotundine methiodide; phase, potassium bromide.

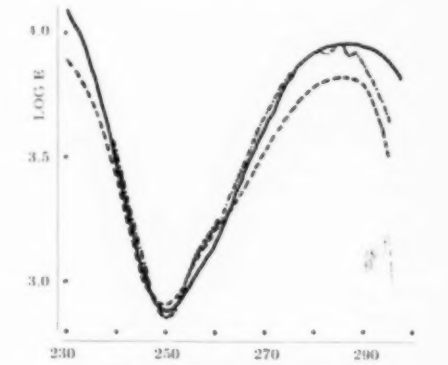


Fig. 3.—Wavelength in mμ.

EXPERIMENTAL

Plant Material.—The above-ground portions of the plant were collected in the late summer of 1954 and 1956 near Nephi, Utah, by Professor Bertrand F. Harrison of the Botany Department of Brigham Young University at Provo, Utah. The plants were identified as *Argemone munita* subsp. *rotundata* by Prof. Gerald B. Ownbey, University of Minnesota, and a voucher specimen was deposited in the University of Minnesota herbarium. The plant material was air-dried and, finally, ground to a 20-mesh fineness for extraction. Preliminary qualitative tests for the presence of alkaloids were positive.

Extraction.—Seven kilograms of powdered plant material was macerated for one hour with alcohol,

[2] In this paper "alcohol" will be taken to mean alcohol U. S. P. XV.

placed in a Lloyd extractor, and continuously extracted for ninety hours. The marc showed the absence of alkaloidal bases when tested qualitatively. The alcoholic extract was concentrated *in vacuo* to remove as much alcohol as possible. Water was added periodically to the extract to replace the alcohol that was removed and this process was continued until the alcohol was completely removed. The aqueous mixture was acidified sufficiently to make it approximately 5% with respect to hydrochloric acid. The acidic solution was removed from the dark sludge, which was worked up several times with additional portions of 5% hydrochloric acid until the sludge was free of alkaloid. All of the acidic extractions were combined and rendered alkaline with saturated sodium carbonate solution. The liberated bases were completely extracted from the aqueous solution by extraction in a separatory funnel with many portions of ether. The combined ethereal extracts were concentrated to a convenient volume and then extracted with 5% hydrochloric acid to obtain an acidic solution of the alkaloidal bases. This acidic solution was then rendered strongly alkaline by the use of a 25% sodium hydroxide solution and extracted completely with ether until the ethereal extracts showed no test for alkaloids although the alkaline aqueous phase gave a strong alkaloidal test. The ethereal extracts were combined, washed, and dried for further examination of the nonphenolic alkaloids. The strongly alkaline solution remaining in the separatory funnel was then converted to a mildly alkaline solution by carbonation through the addition of excess solid carbon dioxide, a process which released the phenolic alkaloids as free bases. These were extracted either with ether or dichloromethane, the latter being preferable, until the aqueous phase failed to show an alkaloidal test. The solution of phenolic bases in the immiscible solvent was then washed with water and dried over anhydrous sodium sulfate.

Nonphenolic Fraction.—The ethereal solution of nonphenolic bases was distilled under reduced pressure to yield 1.2 Gm. of a dark brown residue, corresponding to a yield of 0.015% based on the dry weight of the crude plant material. The crude alkaloidal residue was very soluble in dilute acid, acetone, and methanol but insoluble in dilute base, water, benzene, and petroleum ether. Attempts to crystallize the crude material were unsuccessful. The material (600 mg.) was then chromatographed on an alumina column (18 Gm.) and eluted with benzene-ether mixtures (from 3:1 to 1:3), and finally with pure ether. As a result of the chromatographic procedure, an almost colorless residue was obtained giving a strong alkaloidal test and weighing 220 mg. The dark brown color remained on the column and apparently was nonalkaloidal because, when washed off with methanol, it failed to give an alkaloidal test. A small portion of the alkaloidal residue from the eluted fraction was dissolved in alcohol and treated with an excess of methyl iodide but failed to yield a methiodide even on refrigeration.[3]

Another small portion of this material was treated with a drop of concentrated sulfuric acid and found to give a lavender-colored solution which quickly turned to orange. The remainder (200 mg.) of the alkaloidal fraction was rechromatographed on aluminum oxide (6 Gm.) with chloroform as the eluant. Two fractions were obtained, the first consisting of approximately 60 mg. and the latter of about 100 mg. Although the first fraction was able to be recrystallized from Skelly B, the amount of material became vanishingly small and the best melting point recorded was 125–134°. The second fraction was in the form of a pink amorphous powder which resisted recrystallization and failed to give a methiodide from an alcoholic solution. Because of the small amounts of material in the nonphenolic fraction, the work on this material was discontinued.

Phenolic Fraction.—The ether (or dichloromethane) solution of the phenolic alkaloids was concentrated under reduced pressure to yield a yellowish-white semicrystalline residue representing 0.067% of the crude plant material taken. A portion of this residue was seemingly quite soluble in alcohol whereas a crystalline white residue remained which was considerably more resistant to solution in alcohol. Although a portion of the residue went into solution with alcohol, the majority of the phenolic fraction was in the difficultly soluble residue and it was later found that even the material that went into alcohol so readily was largely of the less soluble type when freed of contaminate. We have not been able to find another alkaloid in this fraction although the conventional techniques of crystallization and chromatography have been employed. The best solvent for recrystallization of the white, crystalline residue was found to be methanol with repeated recrystallizations yielding glistening, white leaflets, m. p. 245–245.5°.[4]

The crystalline material is soluble in dilute acid, dilute sodium hydroxide, difficultly soluble in methanol, ethanol, and chloroform and insoluble in acetone, water, and dilute sodium carbonate solution. The ascending paper chromatographic technique of Levine and Fischbach (13), as modified for slow moving alkaloids, was used to determine if more than one alkaloid were present. Repeated experiments showed only one alkaloid with an R_f value of 0.29. The alkaloid appears to be a new one and has provisionally been named "rotundine" after the subspecies of the plant.

Rotundine.—A highly purified sample of rotundine was dried *in vacuo* at 100° in an Abderhalden drying pistol employing phosphorus pentoxide as a desiccant for three hours. The sample lost no weight so it was assumed that solvent of crystallization was probably absent. The specific rotation of rotundine was found to be $[\alpha]_D^{25} = -265.8°$ (c = 0.158) in methanol.

Anal.[5]—Calcd. for $C_{19}H_{19}(OCH_3)_2(OH)_2(NCH_3)$: C, 69.73; H, 6.59; N, 4.28; —OCH_3, 18.96; —NCH_3, 4.59; Active H, 0.61 (Zerewitinoff). Found: C,

[3] This behavior differs markedly from that of argemonine, which readily forms a methiodide in alcoholic solution even in the presence of other contaminating bases. For this reason, it might be suspected that argemonine is absent from this fraction.

[4] All melting points were determined with the Kofler apparatus.

[5] The analyses in this work were performed by Clark Microanalytical Laboratories at Urbana, Ill., Weiler and Strauss Microanalytical Laboratory at Oxford, England, and the Microanalytical Laboratory in the Chemistry Department, University of Minnesota.

70.02; H, 6.31; N, 4.30; —OCH_3, 19.22; —NCH_3, 3.31; Active H, 0.55.

For the above formula, the calculated molecular weight was 327. An ebullioscopic molecular weight determination using methanol as the solvent showed an experimental value of 323. The use of ethyl methyl ketone as a solvent gave a value of 497, indicating the possible combination of the solvent into a molecular complex with the alkaloid [hemiketal(?)].

An alcoholic solution of rotundine imparted an emerald green color when treated with a few drops of 5% ferric chloride solution.

Methylation of Rotundine.—Rotundine (0.2 Gm.) was dissolved in approximately 25 ml. of absolute methanol and to this was added a fivefold molar excess of diazomethane in ether maintaining a detemperature of not over 5° in the reaction mixture. The solution was allowed to stand at this temperature for two hours and then allowed to come to room temperature slowly. The excess diazomethane and ether were removed with gentle heat and the methanol was removed under an air stream. The amorphous residue was then dissolved in 25 ml. of 5% hydrochloric acid and the acidic solution made strongly alkaline with 25% sodium hydroxide solution. The alkaline solution was then thoroughly extracted with ether to remove the nonphenolic base. The ether extracts were dried over anhydrous sodium sulfate and the ether removed by gentle heating. The residue was recrystallized from a dilute alcoholic solution to finally give prismatic crystals, m. p. 130–135° (with effervescence) (capillary). The crystals were pulverized in a mortar and then dried for four hours at 100° in an Abderhalden drying pistol charged with phosphorus pentoxide. The dried alkaloidal base melted sharply at 151–151.5° (capillary) and a mixed melting point determination with an authentic sample of argemonine failed to depress the melting point.

The methylated rotundine (0.1 Gm.) was converted to the picrate in the usual manner and recrystallized several times from boiling water, m. p. 241–242° (capillary). A mixed melting point in a capillary with an authentic sample of argemonine picrate (9) failed to depress the melting point.

Methylated rotundine (0.1 Gm.) was also converted to the styphnate in the usual manner and recrystallized several times from boiling water, m. p. 247–248° (capillary). A mixed melting point with authentic argemonine styphnate failed to depress the melting point.

Methylated rotundine (0.22 Gm.) was dissolved in 5 ml. of alcohol and treated with 2 ml. of methyl iodide. A crystalline deposit of the methiodide formed within a few minutes. This was removed by filtration and recrystallized from alcohol several times, m. p. 273–274° (decompn.). A mixed melting point with authentic argemonine methiodide failed to depress the melting point. The infrared spectra of the two methiodides was also compared (see Fig. 2) and found to be identical in all respects.

Ultraviolet.—Highly purified samples of rotundine, argemonine, and norargemonine (9) were dissolved in alcohol and suitable dilutions made in order that their characteristic absorption curves in the ultraviolet range could be ascertained. Both the conventional Beckman DU instrument and the automatically recording Cary instrument were utilized, both giving virtually identical curves. All three of the alkaloids showed a minimum at 250 mμ and the maxima were found to be 288, 287, and 287 mμ, respectively, for rotundine, norargemonine, and argemonine (see Fig. 3). The curves were essentially identical although argemonine showed well-defined shoulders at 280.2 and 291.2 mμ.

Pharmacological Testing.[6]—The predominant effect in mice is a slight to moderate decrease in motor activity with an oral dose of 500 mg./Kg. On the same mice, death occurred with an oral dose of 2,000 mg./Kg., but even at these large doses no significant biological activity was observed. No change in mean arterial blood pressure of the anesthetized cat was observed after acute intravenous doses of 20 mg./Kg. or 39 mg./Kg. cumulative doses. No changes in the autonomic nervous system were observed. In general, even with the weak central nervous system depressant effect observed in mice, the action of the alkaloid was considered to be so slight that its value as a possible therapeutic agent was considered very unlikely.

REFERENCES

(1) Charbonnier, L. *J. Pharm. Chem.*, **7**, 348(1868).
(2) Schlotterbeck, J. O., *Proc. Am. Pharm. Mfrs. Assoc.*, **49**, 247(1901).
(3) Santos, A. C., and Adkilen, P., *J. Am Chem. Soc.*, **54**, 2923(1932).
(4) Almeida Costa, O. de, *Bol. assoc. brasil. pharm.*, **14**, 489(1933); through *Chem. Abstr.*, **28**, 1811(1934).
(5) Almeida Costa, O. de, *Rev. flora med. Rio de Janeiro*, **1**, 271(1935); through *Chem. Abstr.*, **29**, 4901(1935).
(6) Slavikova, L., and Slavik, J., *Chem. Listy*, **49**, 1546 (1955), through *Chem. Abstr.*, **50**, 4990(1956).
(7) Foote, P. A., THIS JOURNAL, **21**, 246(1932).
(8) Lazurevskii, G. V., and Sadykov, A., *Trudy Uzbekskogo Gosudarst. Univ., Sbornik Trudov Khim.*, **15**, 182 (1939); through *Chem. Abstr.*, **35**, 4154(1941).
(9) Soine, T. O., and Gisvold, O., THIS JOURNAL, **33**, 185 (1944).
(10) Schermerhorn, J. W., and Soine, T. O., *ibid.*, **40**, 19(1951).
(11) Ownbey, G. B., *Memoirs of the Torrey Botanical Club*, **21**, 67(1958).
(12) Batra, K. V., and Soine, T. O., unpublished study.
(13) Levine, J., and Fischbach, H., THIS JOURNAL, **44**, 43(1955).

[6] The authors are indebted to the Smith Kline and French Laboratories, Philadelphia, Pa., for testing rotundine for any significant pharmacological effects. We are especially indebted to Dr. Robert F. Raffauf for handling the details of our requests for assistance.

Preparation and Properties of New Gastric Antacids I*

Aluminum Hydroxide–Magnesium Carbonate Dried Gels

By STEWART M. BEEKMAN

The preparation and properties of two new, highly reactive aluminum hydroxide–magnesium carbonate dried gels (AHMC) are described. The rate of reaction with gastric acid is shown to be rapid as well as prolonged in the optimum pH range of 3 to 5. Three methods were used to determine reaction rate including the Reheis and Mutch reaction velocity tests as well as a modified procedure of Holbert, Noble, and Grote. An automated apparatus for antacid evaluation is described. Data on thirteen antacid chemicals as well as thirty of the most widely used antacids in liquid and tablet form are presented. The *in vitro* antacid properties of AHMC dried gels compares favorably with reactive liquid aluminum hydroxide gel. The aging characteristics are shown to be excellent.

SINCE ITS INTRODUCTION into therapeutics in 1934 by Einsel, Adams, and Myers (1), gelatinous liquid aluminum hydroxide gel, alone or in effective combination with other antacids such as magnesium hydroxide or magnesium trisilicate, has become the antacid of choice for the medical management of peptic ulcer and gastric hyperacidity. The literature is replete with reports of its successful use in the clinical treatment of these disorders (2–18). *In vitro* antacid evaluation tests carried out by many investigators (19–26) have shown that liquid aluminum hydroxide gel of proved clinical effectiveness yields both prompt and prolonged activity in the optimum pH range of 3 to 5. It also maintains this activity during a long shelf life.

In contrast with the ideal behavior of the liquid product, dried aluminum hydroxide has been shown (24, 27) to be slow in reacting with gastric strength acid, markedly inhibited in that action by pepsin, and to exhibit a diminution in reaction rate on aging.

This paper and others to follow will present *in vitro* antacid evaluation data on several new aluminum hydroxide gels in dry form which compare favorably in their antacid action with liquid aluminum hydroxide gel. The new dried gels have been designed to exhibit (*a*) promptness of reaction with gastric strength acid, (*b*) prolonged reactivity in the pH range of 3 to 5 in the presence of pepsin, (*c*) minimum loss of reactivity on aging, and (*d*) to be made generally available at moderate cost. The variety of dried gels to be made available will enable the pharmaceutical manufacturer to select a product which best meets his requirement.

This paper will describe two new aluminum hydroxide–magnesium carbonate dried gels (AHMC) which are designated as type F-MA11 and type F-MA12. They differ only in the mole ratio of alumina to magnesia present which is 2:1 and 1.25:1, respectively. When a highly reactive and stable aluminum hydroxide gel is intimately blended with a freshly prepared, low temperature, magnesium carbonate gel and the whole carefully reduced to dry form as by spray drying, the resulting white, free flowing, dense, tasteless powder is found to rehydrate readily and react rapidly with gastric strength acid containing pepsin.

EXPERIMENTAL

The average composition and physical properties of the two new AHMC dried gels are shown in Table I.

TABLE I.—ANALYSES OF TYPICAL SAMPLES OF AHMC DRIED GEL

	F-MA11	F-MA12
Aluminum oxide (Al_2O_3)	42.0%	37.1%
Magnesium oxide (MgO)	8.3%	11.7%
Carbonated (CO_3)	20.0%	16.2%
pH 4% aqueous suspension	9.2	9.1
Acid consuming capacity, (ml. 0.1 *N* HCl per Gm.)	287	286
Al_2O_3:MgO Mole Ratio	2:1	1.25:1
Apparent density (cc./Gm.)	0.44	0.2 or 0.4[a]

[a] Depending on method of drying.

X-ray Diffraction Studies.—X-ray diffraction studies were made using the Debye-Scherrer powder technique with thin-wall glass capillaries 0.30 mm. O. D. and 0.25 mm. I. D. The exposures were made for two hours at 35 mv. and 20 ma. on the X-ray tube. Exposures were made of aluminum hydroxide dried gel, magnesium carbonate gel dried, the new AHMC dried gels, and a dry blend of aluminum hydroxide dried gel with magnesium carbonate powder. Aluminum hydroxide dried gel and AHMC dried gels gave no pattern, indicating that

* Received September 2, 1959, from the Research Laboratory, Reheis Co., Inc., Berkeley Heights, N. J.
Presented to the Scientific Section, A. Ph. A. Cincinnati meeting, August 1959.

they are essentially, if not wholly, amorphous. The dry blend gave a faint pattern with lines corresponding to magnesium carbonate. The dry magnesium carbonate gave a very distinct pattern.

Infrared Spectra.—Rock salt infrared spectra (2 to 15 μ) were made on samples of AHMC dried gel as well as each component dried separately. Nujol as well as hexachlorobutadiene (HCBD) mulls were made. A careful examination of the absorption bands of the two components showed that they were present in the absorption spectra of AHMC with no new bands present in the latter. It was concluded that AHMC dried gels are simple mixtures and not new compounds. The infrared spectrum of magnesium carbonate showed that the material chemically is intermediate between $MgCO_3$ and $3\ MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$.

Antacid Activity.—Four principal *in vitro* methods are used to evaluate antacids in our laboratories as follows: (*a*) acid consuming capacity, (*b*) Reheis reaction velocity test, (*c*) Mutch reaction velocity test, and (*d*) procedure of Holbert, Noble, and Grote modified.

Acid Consuming Capacity.—This measures the volume of 0.1*N* HCl with which 1 Gm. of sample may react at 37.5° for one hour with an excess of acid present. The excess acid is back titrated to the Toepfers reagent end point (pH 3.5). Since this test does not show rate of change and since it employs an excess of acid throughout, it has been classified as generally unsatisfactory (21, 28–30). Values obtained for various antacid chemicals are shown in Table II.

TABLE II.—ACID CONSUMING CAPACITY OF VARIOUS ANTACIDS

Sample	ml. 0.1 *N* HCl/Gm. Sample
AHMC type F-MA11	287
AHMC type F-MA12	286
Aluminum dihydroxy aminoacetate	175
Aluminum dihydroxy sodium carbonate	267
Sodium polyhydroxy aluminum monocarbonate hexitol complex	275
Aluminum hydroxide dried gel	267
Sodium bicarbonate	120
Magnesium carbonate	220
Calcium carbonate	210
Magnesium trisilicate	115
Magnesium oxide	495

Reheis Reaction Velocity Test.—This test has been used by the author in our laboratories for eighteen years as a relatively quick method of assessing reaction rates of alumina gels. The weight of sample containing the equivalent of 0.5 Gm. Al_2O_3 is added to 100 ml. 0.1*N* HCl at 37.5° and the whole shaken until the pH rises to about 3.5 (Toepfers reagent). The time is noted and is that required for the sample to react with 34% of the theoretical amount of acid.

A highly reactive alumina gel gives values of ten to twenty seconds. A newly prepared sample of spray dried gel may react in one hundred and twenty seconds and two hundred and forty seconds after three months storage at room temperature. After four years of storage the time may increase to six hundred and sixty seconds. By contrast, samples of AHMC yield values of twenty seconds when new and similar values after two years aging at room temperature. Typical values for various aluminum-containing antacids are shown in Table III.

TABLE III.—REHEIS REACTION VELOCITY VALUES FOR VARIOUS ALUMINUM ANTACIDS

Sample	Seconds
Aluminum hydroxide gel U. S. P	15
Aluminum hydroxide dried gel U. S. P	240
AHMC type F-MA11	22
AHMC type F-MA12	20
Sodium polyhydroxy aluminum monocarbonate hexitol complex	35
Aluminum dihydroxy aminoacetate	29
Aluminum dihydroxy sodium carbonate	15
Aluminum hydroxide dried gel U. S. P.-magnesium carbonate U. S. P. dry blend (4:1)	247

Mutch Reaction Velocity Test.—This test which was described by Mutch (31) in 1946 is similar to the Reheis reaction velocity test described above except that it uses 78% of the theoretical amount of acid per weight of alumina. The time is that required for the sample containing the equivalent of 0.10 Gm. $Al(OH)_3$ to react with 30 ml. 0.1*N* HCl at 37.5° to the Toepfers reagent end point. Typical values for various aluminum-containing chemicals are shown in Table IV.

TABLE IV.—MUTCH REACTION VELOCITY VALUES FOR VARIOUS ALUMINUM ANTACIDS

Sample	Seconds
Aluminum hydroxide gel U. S. P.	40
Aluminum hydroxide dried gel U. S. P.	1,080
AHMC type F-MA11	54
AHMC type F-MA12	50
Sodium polyhydroxy aluminum monocarbonate hexitol complex	275
Aluminum dihydroxy aminoacetate	103
Aluminum dihydroxy sodium carbonate	70
Aluminum hydroxide dried gel U. S. P.-magnesium carbonate U. S. P. dry blend (4:1)	1,570

Procedure of Holbert, Noble, and Grote Modified.—In 1948 Holbert, Noble, and Grote (28) published an *in vitro* method which was adapted from the carefully worked-out procedure of Johnson and Duncan (21). This made use of the concept of representing the secretion of fresh gastric juice and also continuous loss of gastric juice–antacid mixture through the pylorus. Holbert, *et al.*, added a 2-Gm. dose of antacid to 150 ml. of artificial gastric juice at 37.5° with constant agitation, and after ten minutes withdrew 20 ml. of mixture and replaced it with 20 ml. of fresh artificial gastric juice. This withdrawal and replacement was carried out periodically every ten minutes until the pH fell below 3.5. Murphey (27) also used this method with an artificial gastric juice consisting of 2.0 Gm. pepsin N. F. per liter of pH 1.5 hydrochloric acid.

We have adapted this method with the following changes which we feel impart a higher degree of precision and insures a greater reproducibility of results.

A dose of antacid is added to the equivalent of 150 ml. artificial gastric juice at 37.5 ± 1° contained in a

jacketed, baffled, glass vessel provided with a glass and reference electrode, thermocompensator, agitator, and overflow device. The artificial gastric juice is pH 1.5 hydrochloric acid (0.0316 *N*) containing 2.0 Gm. pepsin N. F. per liter. After ten minutes, additional gastric juice at 37.5 ± 1° is added continuously at the equivalent of 120 ml. per hour by means of a Milton Roy controlled volume "mini pump." A jacketed, 3-gallon glass reservoir of artificial gastric juice is maintained at 37.5° by means of an Aminco constant temperature water circulating bath. Excess gastric juice–antacid mixture is allowed to flow continuously from the glass reactor at the equivalent of 120 ml. per hour.

The apparatus designed for this purpose is shown in Figs. 1 and 2. The pH is measured by means of a Beckman model W pH meter which is connected to a Weston strip chart recorder operating with a chart speed of six inches per hour. The pH meter indicates and records values between 2.0 and 12.0. In practice, the actual amounts and sizes are four times those stated above to avoid crowding into a small container. Thus, 600 ml. of artificial gastric juice are added to a jacketed, 1-liter, glass vessel provided with an overflow tube at the 650 ml. level. The agitator is a two-inch, three-bladed, marine type propeller which operates at exactly 400 r. p. m. Glass baffles prevent swirling in the reaction vessel. The pumping rate for artificial gastric juice is 480 ml.

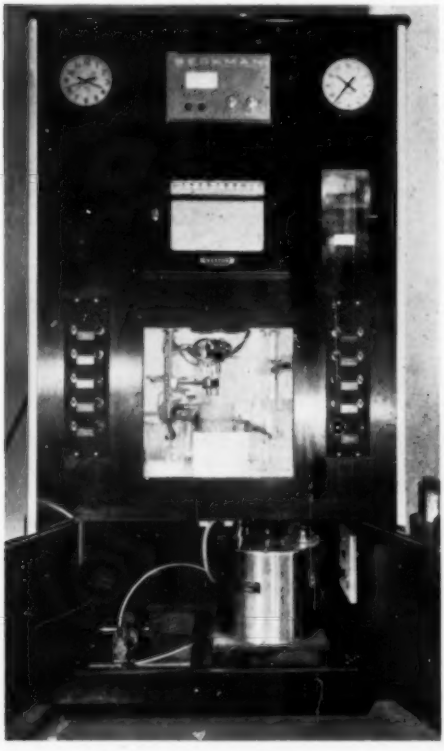


Fig. 1.—Automated apparatus for *in vitro* evaluation of antacid activity.

Fig. 2.—Close-up of cubicle showing details of glass reaction cell.

± 24 ml. per hour. The overflow is also at 480 ml. per hour. The sample size is four times the dose size. After addition of the sample to the glass reaction cell, the interval timer is started and pH values manually recorded every minute up to ten minutes, after which the constant addition of gastric juice is started. With the automation provided, the apparatus needs no further attention until the run is completed, which is usually when the pH has gone below 3.0. The average run is one hundred and twenty minutes. The reproducibility of results is very high.

When evaluating dry powders, 2, 4, or 8 Gm. (corresponding to doses of 0.5, 1, and 2 Gm., respectively) are dispersed in 35 ml. of water and poured in at time zero. Sucking tablets are ground to 100% through 100 mesh before dispersing in 35 ml. of water. Disintegrating types of tablets are dropped into 35 ml. of water and allowed to hydrate and disintegrate before adding. Liquid antacids are added directly to the glass reaction cell.

More than 800 two-hour evaluations have been made since late 1955 including countless samples which have been submitted by various pharmaceutical manufacturers. Many samples were blind samples to insure complere objectivity. Results have been treated on a highly confidential basis. Some results obtained on various antacid chemicals are shown in Table V and plotted in Figs. 3–15. Results obtained on the most widely used liquid commercial antacids are shown in Table VI, and on tablet preparations in Table VII.

Effect of Pepsin.—To demonstrate the effect of pepsin in artificial gastric juice on the antacid activity of dried aluminum hydroxide gel U. S. P. as measured by the modified procedure of Holbert, Noble, and Grote, runs were made on 1.0- and 2.0-Gm. samples with and without pepsin. The data are shown in Table VIII and Fig. 16.

Liquid aluminum hydroxide gel is not inhibited in this manner by pepsin. The full significance of this fact is not yet understood. AHMC dried gels do not show such inhibition by gastric pepsin.

Effect of Age.—The antacid activity of four-year old samples of AHMC dried gel, aluminum dihydroxy aminoacetate, and aluminum dihydroxy sodium carbonate was determined by the Holbert, Noble, and Grote method on the basis of 1-Gm. doses.

TABLE V.—*In Vitro* EVALUATION OF ANTACID ACTIVITY OF VARIOUS ANTACID CHEMICALS[a,b]

Time (min.)	1				2		3			4			5		
	5 ml.	10 ml.	12.5 ml.	15 ml.	1 Gm.	2 Gm.	0.5 Gm.	1.0 Gm.	2.0 Gm.	0.5 Gm.	1.0 Gm.	2.0 Gm.	0.5 Gm.	1.0 Gm.	2.0 Gm.
0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
1	2.6	3.8	3.9	3.9	2.0	2.2	2.7	4.0	4.1	2.4	3.5	4.4	2.1	3.0	3.8
2	3.8	3.9	3.9	4.0	2.0	2.3	3.6	4.1	4.3	2.8	3.9	4.5	2.7	3.7	4.0
3	3.9	3.9	4.0	4.0	2.1	2.4	3.8	4.2	4.3	3.2	4.0	4.7	3.2	3.9	4.1
4	3.9	3.9	4.0	4.1	2.1	2.4	3.9	4.2	4.4	3.7	4.1	4.8	3.4	3.9	4.1
5	3.9	3.9	4.0	4.1	2.1	2.5	4.0	4.2	4.4	4.0	4.1	4.9	3.6	4.0	4.1
10	3.9	3.9	4.0	4.2	2.3	3.3	4.1	4.3	4.5	4.2	4.2	5.3	4.0	4.2	4.2
20	3.9	3.9	4.0	4.2	2.9	4.0	4.1	4.3	4.4	4.2	4.2	5.1	4.0	4.2	4.2
30	3.9	3.9	4.0	4.2	3.9	4.0	4.1	4.3	4.4	4.2	4.2	4.8	4.0	4.1	4.2
40	3.9	3.9	4.0	4.2	3.9	4.0	4.1	4.2	4.3	4.2	4.2	4.7	3.8	4.1	4.2
50	3.9	3.9	4.0	4.2	3.8	4.0	4.0	4.2	4.3	4.2	4.1	4.5	3.6	4.0	4.2
60	3.8	3.9	4.0	4.1	3.8	4.0	4.0	4.2	4.3	4.1	4.1	4.5	3.3	4.0	4.2
70	3.5	3.9	4.0	4.1	3.7	4.0	3.9	4.2	4.3	3.9	4.1	4.4	3.0	3.9	4.2
80	3.0	3.9	4.0	4.1	3.5	3.9	3.7	4.2	4.3	3.6	4.0	4.3	2.8	3.8	4.2
90	2.6	3.8	3.9	4.1	3.2	3.9	3.5	4.1	4.2	3.2	4.0	4.3	2.7	3.6	4.1
100	2.4	3.8	3.9	4.1	3.0	3.9	3.1	4.1	4.2	2.9	4.0	4.3	2.5	3.4	4.1
110	2.2	3.7	3.9	4.1	2.8	3.8	2.8	4.1	4.2	2.7	3.9	4.2	2.4	3.2	4.1
120	2.1	3.4	3.8	4.1	2.6	3.7	2.6	4.0	4.2	2.5	3.9	4.2	2.3	3.0	4.0

Time min.	6			7			8		9			10		
	0.5 Gm.	1.0 Gm.	2.0 Gm.	0.5 Gm.	1.0 Gm.	2.0 Gm.	0.5 Gm.	1.1 Gm.	0.5 Gm.	1.0 Gm.	2.0 Gm.	0.5 Gm.	1.0 Gm.	2.0 Gm.
0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
1	3.1	3.8	4.2	2.0	2.0	2.3	2.3	3.6	2.0	2.0	2.0	6.6	7.2	7.6
2	3.5	4.0	4.3	2.0	2.0	2.5	2.9	3.8	2.0	2.2	2.3	6.9	7.6	7.8
3	3.6	4.1	4.4	2.0	2.0	2.7	3.5	3.9	2.0	2.6	2.9	7.1	7.8	7.9
4	3.7	4.1	4.5	2.0	2.1	2.9	3.7	4.0	2.0	4.1	4.2	7.2	7.9	8.0
5	3.8	4.2	4.5	2.1	2.2	3.0	3.7	4.0	2.6	5.3	5.7	7.8	8.0	8.0
10	3.9	4.3	4.9	2.4	2.5	3.2	3.9	4.0	2.2	6.8	7.2	7.2	7.7	7.8
20	3.6	4.2	4.6	2.5	2.8	3.2	3.8	4.0	2.4	6.7	7.2	7.6	7.6	7.8
30	3.2	4.1	4.3	2.3	2.8	3.1	3.7	4.0	2.4	6.6	7.1	7.0	7.6	7.8
40	3.1	4.0	4.3	2.3	2.7	3.0	3.7	4.0	2.3	6.3	7.1	6.8	7.6	7.7
50	3.0	3.9	4.2	2.3	2.7	3.0	3.6	3.9	2.2	5.8	7.0	6.5	7.5	7.7
60	2.9	3.9	4.2	2.2	2.6	2.9	3.5	3.9	2.1	4.8	7.0	6.2	7.4	7.7
70	2.8	3.8	4.2	2.1	2.6	2.9	3.2	3.9	2.1	3.9	6.9	5.3	7.3	7.7
80	2.6	3.8	4.1	2.1	2.5	2.8	2.8	3.8	2.1	3.2	6.7	3.0	7.1	7.6
90	2.4	3.7	4.1	2.1	2.4	2.7	2.5	3.8	2.0	2.7	6.5	2.6	6.9	7.5
100	2.3	3.6	4.0	2.0	2.3	2.7	2.3	3.7	2.0	2.4	6.3	2.4	6.7	7.5
110	2.2	3.6	4.0	2.0	2.3	2.6	2.2	3.5	2.0	2.3	5.6	2.3	6.4	7.4
120	2.1	3.5	4.0	2.0	2.2	2.5	2.1	3.4	2.0	2.2	5.6	2.2	5.8	7.3

Time, min.	11			12			13		
	0.5 Gm.	1.0 Gm.	2.0 Gm.	0.5 Gm.	1.0 Gm.	2.0 Gm.	0.5 Gm.	1.0 Gm.	2.0 Gm.
0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
1	6.2	8.9	9.1	5.7	5.8	5.9	5.4	6.4	6.9
2	7.2	9.0	9.1	5.8	5.8	6.0	5.5	6.4	7.0
3	8.6	9.0	9.2	5.8	5.8	6.0	5.5	6.5	7.0
4	8.7	9.1	9.2	5.9	5.9	6.0	5.5	6.5	7.0
5	8.8	9.1	9.2	5.9	5.9	6.0	5.5	6.5	7.1
10	8.9	9.1	9.2	6.0	6.0	6.1	5.6	6.7	7.2
20	8.9	8.9	9.1	5.9	6.0	6.1	4.5	6.7	7.3
30	7.8	8.8	9.1	5.9	6.0	6.1	2.8	6.7	7.2
40	7.6	8.8	9.1	5.9	6.0	6.1	2.5	6.6	7.2
50	7.3	8.7	9.1	5.8	6.0	6.1	2.3	6.5	7.2
60	6.9	8.6	9.0	5.7	6.0	6.1	2.2	6.2	7.2
70	6.4	8.5	9.0	4.7	6.0	6.1	2.1	5.3	7.2
80	5.6	8.3	8.9	3.2	5.9	6.1	2.1	2.9	7.1
90	4.9	8.2	8.9	2.8	5.9	6.1	2.0	2.6	7.0
100	4.4	8.0	8.9	2.5	5.8	6.1	2.0	2.6	6.8
110	3.9	7.7	8.8	2.3	5.8	6.1	2.0	2.2	6.5
120	3.2	7.4	8.7	2.2	5.7	6.0	2.0	2.2	6.3

[a] Time DETERMINATION pH at various dose levels, procedure of Holbert, Noble, and Grote modified.
[b] Identification of samples:

1. Aluminum hydroxide gel U. S. P.
2. Aluminum hydroxide dried gel U. S. P.
3. AHMC type F-MA11.
4. AHMC type F-MA12.
5. Aluminum dihydroxy aminoacetate.
6. Aluminum dihydroxy sodium carbonate.
7. Aluminum proteinate.
8. Sodium polyhydroxy aluminum monocarbonate hexitol complex.
9. Magnesium trisilicate U. S. P.
10. Magnesium carbonate U. S. P.
11. Magnesium hydroxide N. F.
12. Calcium carbonate U. S. P.
13. Sodium bicarbonate U. S. P.

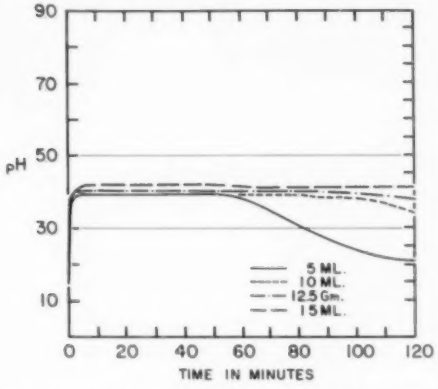


Fig. 3.—Aluminum hydroxide gel U. S. P. Procedure of Holbert, Noble, and Grote modified.

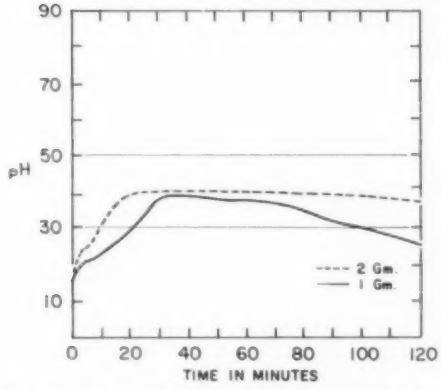


Fig. 4.—Aluminum hydroxide dried gel U. S. P., procedure of Holbert, Noble, and Grote modified.

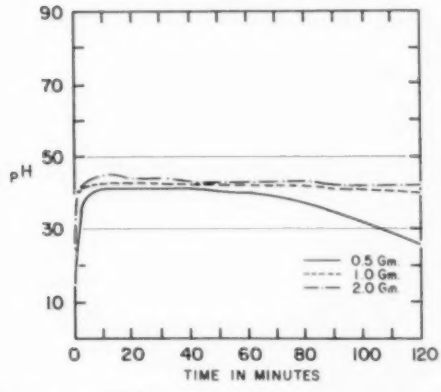


Fig. 5.—AHMC type F-MA11, procedure of Holbert, Noble, and Grote modified.

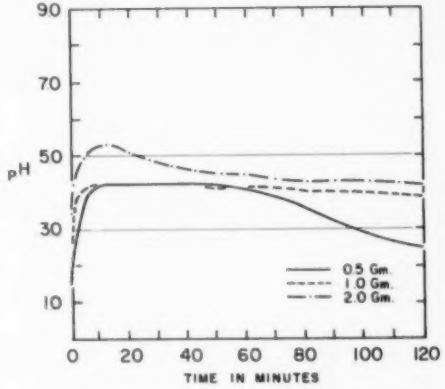


Fig. 6.—AHMC type F-MA12, procedure of Holbert, Noble, and Grote modified.

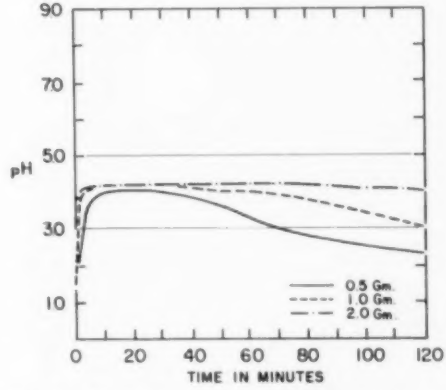


Fig. 7.—Aluminum dihydroxy aminoacetate, procedure of Holbert, Noble, and Grote modified.

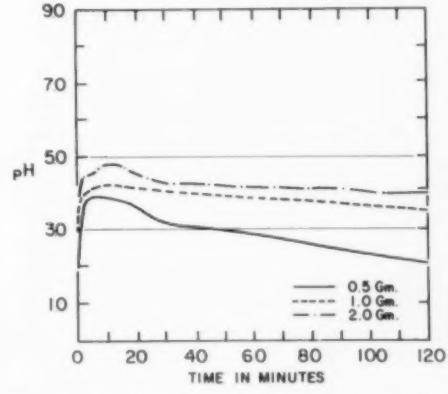


Fig. 8.—Aluminum dihydroxy sodium carbonate, procedure of Holbert, Noble, and Grote modified.

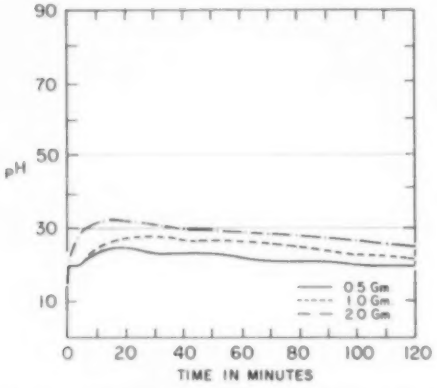


Fig. 9.—Aluminum proteinate, procedure of Holbert, Noble, and Grote modified.

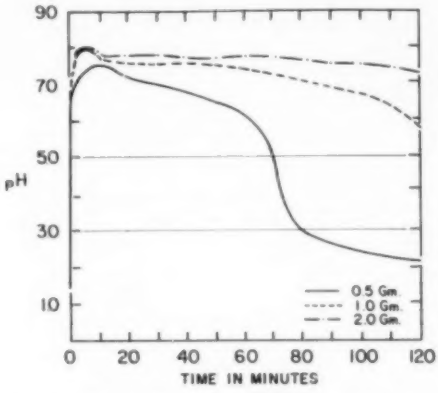


Fig. 12.—Magnesium carbonate U. S. P., procedure of Holbert, Noble, and Grote modified.

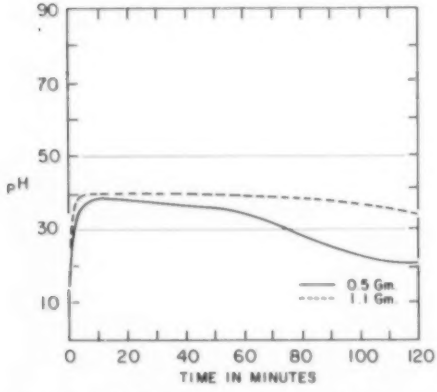


Fig. 10.—Sodium polyhydroxy aluminum monocarbonate hexitol complex, procedure of Holbert, Noble, and Grote modified.

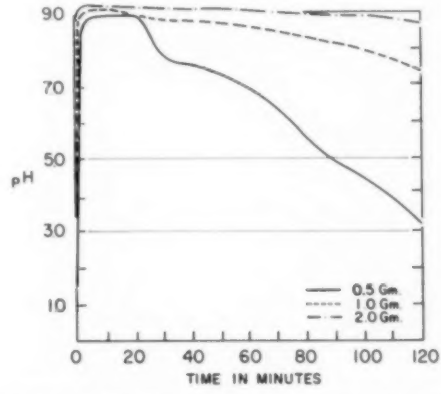


Fig. 13.—Magnesium hydroxide N. F., procedure of Holbert, Noble, and Grote modified.

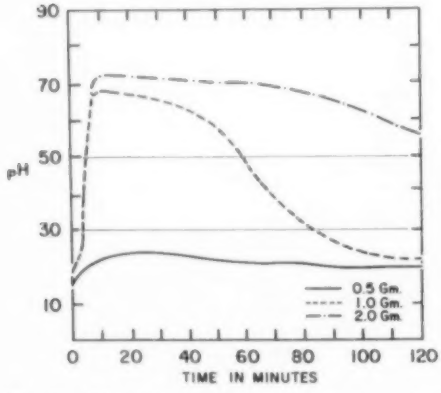


Fig. 11.—Magnesium trisilicate U. S. P., procedure of Holbert, Noble, and Grote modified.

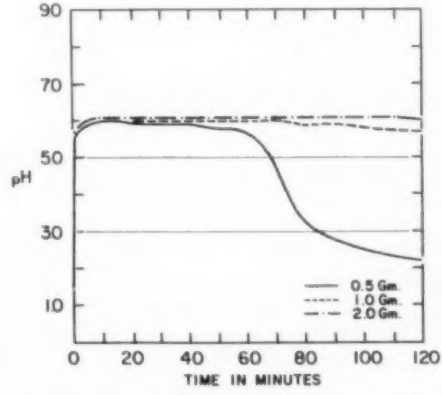


Fig. 14.—Calcium carbonate, procedure of Holbert, Noble, and Grote modified.

TABLE VI.—*In Vitro* EVALUATION OF VARIOUS COMMERCIAL LIQUID ANTACIDS[a, b]

Time, min.	14 15 ml.	15 15 ml.	16 15 ml.	17 4 ml.	17 8 ml.	17 15 ml.	18 15 ml.	19 15 ml.	20 15 ml.	21 5 ml.	21 10 ml.	22 4 ml.	23 25 ml.
0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
1	2.6	4.0	3.9	2.1	3.0	3.8	5.6	4.2	3.9	2.7	3.7	2.0	3.0
2	3.1	4.1	4.0	2.5	3.3	4.0	5.7	4.2	4.0	3.3	3.9	2.0	3.0
3	3.5	4.1	4.1	2.7	3.4	4.1	5.7	4.3	4.0	3.8	4.0	2.0	3.1
4	3.7	4.1	4.1	2.9	3.5	4.2	5.8	4.3	4.0	3.9	4.1	2.0	3.1
5	3.8	4.1	4.1	3.0	3.6	4.3	5.8	4.3	4.1	4.0	4.1	2.0	3.1
10	4.1	4.1	4.2	3.2	3.8	4.6	5.9	4.4	4.1	4.1	4.2	2.0	3.2
20	4.1	4.1	4.2	3.0	3.6	4.3	5.8	4.2	4.1	4.2	4.2	2.0	3.0
30	4.2	4.0	4.2	2.8	3.5	4.1	5.7	4.2	4.0	4.3	4.2	2.0	2.9
40	4.2	4.0	4.3	2.7	3.4	3.9	5.5	4.1	4.0	4.3	4.3	2.0	2.9
50	4.2	4.0	4.3	2.5	3.2	3.7	5.4	4.1	3.9	4.3	4.3	2.0	2.8
60	4.1	4.0	4.3	2.3	3.1	3.6	5.2	4.1	3.9	4.2	4.3	2.0	2.8
70	4.1	3.9	4.3	2.2	2.9	3.5	5.0	4.0	3.8	4.1	4.3	2.0	2.8
80	4.0	3.9	4.3	2.1	2.7	3.4	4.7	4.0	3.6	4.0	4.2	2.0	2.7
90	3.9	3.9	4.2	2.1	2.5	3.4	4.5	4.0	3.5	4.0	4.2	2.0	2.7
100	3.7	3.8	4.0	2.0	2.4	3.3	4.4	3.9	3.5	3.8	4.1	2.0	2.7
110	3.5	3.8	3.9	2.0	2.3	3.3	4.3	3.8	3.3	3.6	4.0	2.0	2.6
120	3.0	3.7	3.8	2.0	2.2	3.2	4.2	3.7	..	3.3	3.9	2.0	2.6

[a] Time *versus* pH at various dose levels, procedure of Holbert, Noble, and Grote modified.
[b] Identification of samples:

14. Aluminum hydroxide gel U. S. P., brand A.
15. Aluminum hydroxide gel U. S. P., brand B.
16. Aluminum–magnesium hydroxide.
17. Aluminum hydroxide (low reactive)–magnesium trisilicate.
18. Aluminum hydroxide with magnesium trisilicate.
19. Magnesium trisilicate (650 mg.) in aluminum hydroxide (322 mg.) per 5 ml.
20. Aluminum hydroxide gel with magnesium hydroxide.
21. Aluminum hydroxide gel–magnesium hydroxide–sorbitol.
22. Bismuth aluminate cream.
23. Aluminum phosphate gel U. S. P.

TABLE VII.—*In Vitro* EVALUATION OF VARIOUS COMMERCIAL ANTACID TABLETS[a, b]

Time, min.	24 1 Tab.	24 2 Tab.	25 1 Tab.	25 2 Tab.	26 1 Tab.	26 2 Tab.	27 1 Tab.	27 2 Tab.	27 4 Tab.	28 1 Tab.	28 2 Tab.	28 4 Tab.	29 1 Tab.	29 2 Tab.	29 4 Tab.
0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
1	2.0	2.0	2.0	2.0	2.2	3.8	2.8	3.5	3.5	2.0	2.7	4.1	2.0	3.9	3.3
2	2.0	2.1	2.0	2.2	3.3	4.0	3.4	3.9	3.8	2.0	3.3	5.0	2.1	4.6	4.4
3	2.0	2.1	2.0	2.4	3.7	4.1	3.6	4.1	3.9	2.4	3.7	5.5	2.3	5.0	4.9
4	2.0	2.2	2.1	2.6	3.8	4.1	3.9	4.1	3.9	2.6	3.8	5.7	2.4	5.4	5.3
5	2.0	2.3	2.1	2.8	3.8	4.1	3.9	4.2	4.0	2.7	4.0	5.9	2.6	5.7	5.7
10	2.4	3.0	2.3	3.4	3.9	4.2	4.0	4.2	4.1	2.9	5.2	6.1	3.2	6.4	6.6
20	3.5	3.7	2.3	3.3	4.0	4.2	3.9	4.2	4.2	2.8	4.6	5.9	2.9	5.0	5.1
30	3.5	3.7	2.3	3.1	4.0	4.2	3.8	4.2	4.2	2.6	4.2	5.7	2.7	4.8	5.0
40	3.5	3.7	2.2	2.9	4.0	4.2	3.6	4.2	4.2	2.4	3.8	5.6	2.6	4.7	4.9
50	3.5	3.7	2.2	2.7	4.0	4.1	3.4	4.2	4.1	2.3	3.4	5.4	2.5	4.5	4.8
60	3.4	3.7	2.1	2.6	4.0	4.1	3.0	4.1	4.1	2.2	3.3	5.2	2.3	4.3	4.6
70	3.4	3.6	2.1	2.4	3.9	4.1	2.8	4.1	4.1	2.2	3.1	4.9	2.3	4.2	4.4
80	3.3	3.5	2.0	2.3	3.7	4.1	2.6	3.9	4.0	2.1	3.0	4.6	2.2	4.0	4.3
90	3.2	3.5	2.0	2.2	3.4	4.1	2.5	3.7	3.9	2.0	2.9	4.1	2.1	3.7	4.2
100	3.1	3.3	2.0	2.1	2.9	4.0	2.3	3.5	3.6	2.0	2.7	3.7	2.1	3.5	4.2
110	3.0	3.1	2.0	2.1	2.6	4.0	2.2	3.2	3.3	2.0	2.6	3.3	2.0	3.2	4.1
120	2.9	3.0	2.0	2.0	2.4	4.0	2.1	2.9	3.1	2.0	2.5	3.0	2.0	2.9	4.0

Time, min.	30 1 Tab.	30 2 Tab.	30 3 Tab.	31 1 Tab.	31 2 Tab.	32 2 Tab.	33 2 Tab.	33 4 Tab.	34 1 Tab.	34 2 Tab.	34 4 Tab.	35 1 Tab.	35 2 Tab.	36 1 Tab.	36 2 Tab.
0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
1	2.0	3.0	3.6	2.0	2.8	2.0	2.8	3.5	2.4	3.6	5.3	3.5	4.0	5.2	5.1
2	2.3	3.8	3.8	2.1	3.4	2.0	3.4	4.0	2.9	3.9	6.2	3.6	4.1	5.3	5.4
3	2.6	3.9	3.9	2.2	3.7	2.1	3.7	4.2	3.2	4.0	6.6	3.7	4.1	5.4	5.5
4	2.7	4.0	4.0	2.3	3.8	2.1	3.9	4.3	3.4	4.0	6.9	3.8	4.2	5.4	5.5
5	3.2	4.0	4.0	2.5	3.9	2.1	4.0	4.5	3.5	4.1	7.1	4.0	4.2	5.5	5.6
10	3.8	4.0	4.0	3.2	4.0	2.5	4.2	6.7	3.8	4.3	7.6	4.1	4.2	5.6	5.7
20	3.6	4.0	4.0	3.4	4.0	2.8	4.3	7.1	3.8	4.4	7.8	3.9	4.1	5.2	5.5
30	3.4	4.0	4.0	3.5	4.0	2.8	4.3	6.8	3.7	4.3	7.7	3.6	4.0	5.2	5.5
40	3.0	4.0	4.0	3.3	4.0	2.8	4.2	6.4	3.6	4.2	7.5	3.3	4.0	5.0	5.5
50	2.6	4.0	3.9	3.1	3.9	2.8	4.1	5.7	3.5	4.0	7.1	3.1	3.9	4.7	5.4
60	2.4	3.9	3.9	2.9	3.9	2.8	4.0	4.9	3.4	3.9	6.5	2.9	3.8	4.2	5.4
70	2.2	3.9	3.9	2.7	3.8	2.7	3.9	4.4	3.3	3.8	5.9	2.7	3.6	3.1	5.3
80	2.1	3.8	3.8	2.6	3.7	2.5	3.8	4.2	3.2	3.8	5.2	2.6	3.5	2.6	5.2
90	2.0	3.6	3.7	2.5	3.6	2.4	3.8	4.0	3.0	3.7	4.6	2.4	3.3	2.4	5.1
100	2.0	3.3	3.7	2.4	3.5	2.4	3.7	3.8	2.9	3.7	4.3	2.3	3.2	2.2	4.8
110	2.0	2.9	3.5	2.3	3.3	2.3	3.6	3.7	2.7	3.6	4.0	2.2	3.0	2.1	4.3
120	2.0	2.7	3.4	2.2	3.1	2.3	3.5	3.6	2.6	3.5	3.8	2.1	2.9	2.1	3.0

TABLE VII (*continued*)

Time, min.	37	38			39		40		41	42	43		
	2	1	2	4	1	2	1	2	2	2	1	2	4
	Tab.	Tabl	Tab.	Tab.	Tab.	Tab.	Tab.	Tab.	Tab.	Tab.	Tab.	Tab.	Tab.
0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
1	6.0	4.5	5.7	6.2	5.5	6.0	5.4	6.0	2.2	3.5	3.9	4.1	4.3
2	6.2	5.1	6.2	7.1	5.7	6.3	5.7	6.2	2.8	3.8	4.0	4.3	4.8
3	6.3	5.4	6.6	7.9	5.8	6.5	5.9	6.4	3.3	3.9	4.1	4.4	5.2
4	6.3	5.6	7.0	8.2	5.9	6.6	6.0	6.4	3.9	3.9	4.1	4.6	5.4
5	6.4	5.7	7.5	8.3	6.0	6.7	6.1	6.5	4.4	3.9	4.1	4.7	5.6
10	6.5	6.1	8.2	8.6	6.2	7.1	6.3	6.7	5.5	4.0	4.1	5.1	5.8
20	6.3	5.7	7.9	8.4	6.9	6.1	6.3	6.5	5.2	4.0	4.0	4.5	4.9
30	6.2	5.0	7.6	8.3	5.9	6.7	6.2	6.5	4.5	4.0	3.9	4.3	4.8
40	6.2	4.3	7.1	8.1	5.8	6.4	6.1	6.5	3.9	4.0	3.9	4.2	4.7
50	6.2	3.6	6.7	7.9	5.7	6.3	6.0	6.5	3.5	3.9	3.8	4.1	4.5
60	6.2	3.0	6.3	7.7	5.4	6.1	5.7	6.4	3.3	3.9	3.6	4.1	4.4
70	6.1	2.6	5.9	7.5	4.9	6.0	4.9	6.4	2.9	3.9	3.2	4.0	4.3
80	6.0	2.5	5.4	7.2	4.6	6.0	4.0	6.3	2.7	3.8	2.8	4.0	4.2
90	5.9	2.3	4.7	7.0	4.1	5.8	3.7	6.2	2.5	3.7	2.5	3.9	4.2
100	5.7	2.2	4.0	6.7	3.6	5.7	3.5	6.1	2.4	3.5	2.3	3.8	4.1
110	5.3	2.2	3.3	6.5	3.1	5.5	3.3	5.8	2.3	3.3	2.2	3.7	4.0
120	4.2	2.1	2.9	6.2	2.8	5.2	3.1	5.1	2.2	3.0	2.1	3.5	4.0

[a] Time *versus* pH at various dose levels, procedure of Holbert, Noble, and Grote modified.

[b] Identification of samples:

24. Aluminum hydroxide dried gel (660 mg.).
25. Aluminum hydroxide dried gel (660 mg.).
26. AHMC type F-MA11 (660 mg.).
27. Aluminum dihydroxy aminoacetate (500 mg.).
28. Magnesium trisilicate (500 mg.) and aluminum hydroxide (250 mg.).
29. Aluminum-magnesium hydroxide (400 mg.).
30. Sodium polyhydroxy aluminum monocarbonate hexitol complex (320 mg.) calculated as aluminum hydroxide dried gel U. S. P.
31. Aluminum hydroxide (648 mg.) and magnesium trisilicate (324 mg.).
32. Aluminum hydroxide and magnesium trisilicate.
33. Aluminum hydroxide-glycine (450 mg.), magnesium oxide (60 mg.), and bellafoline (0.5 mg.).
34. Aluminum hydroxide-glycine (450 mg.) and magnesium oxide (60 mg.).
35. Aluminum dihydroxy sodium carbonate (330 mg.).
36. Calcium carbonate (510 mg.), magnesium carbonate (30 mg.), and magnesium trisilicate (40 mg.).
37. Calcium carbonate, magnesium carbonate, and magnesium trisilicate.
38. Calcium carbonate, magnesium trisilicate, and magnesium hydroxide.
39. Calcium carbonate, magnesium carbonate, and magnesium trisilicate.
40. Heavy magnesium carbonate (182 mg.), light magnesium carbonate (24 mg.), calcium carbonate (441 mg.), and aluminum hydroxide (223 mg.).
41. Regenol (100 mg.), magnesium trisilicate (100 mg.), aluminum hydroxide dried gel (90 mg.), calcium carbonate (100 mg.), magnesium carbonate (100 mg.), and egraine (45 mg.).
42. AHMC type F-MA11 (330 mg.) and activated attapulgite (130 mg.).
43. Sodium polyhydroxy aluminum monocarbonate hexitol complex (320 mg.), calculated as aluminum hydroxide dried gel U. S. P., and magnesium hydroxide (75 mg.).

TABLE VIII.—EFFECT OF PEPSIN ON ANTACID ACTIVITY OF DRIED ALUMINUM HYDROXIDE GEL U. S. P., TIME *versus* pH

Time, min.	Concentration of Pepsin			
	2.0 Gm./L.		0.0 Gm./L.	
	1 Gm.	2 Gm.	1 Gm.	2 Gm.
0	1.5	1.5	1.5	1.5
1	2.0[a]	2.1	2.0[a]	2.2
3	2.0[a]	2.2	2.0[a]	2.4
5	2.0[a]	2.3	2.0	2.5
10	2.0	2.6	3.9	4.2
20	2.2	3.3	4.1	4.2
30	2.4	4.0	4.2	4.2
40	2.6	4.0	4.2	4.2
50	2.7	4.0	4.2	4.2
60	2.8	4.0	4.2	4.2
70	2.8	4.0	4.2	4.2
80	2.7	3.9	4.2	4.2
90	2.6	3.9	4.2	4.2
100	2.6	3.8	4.2	4.2
110	2.5	3.7	4.2	4.1
120	2.4	3.5	4.1	4.1

[a] Indicates less than.

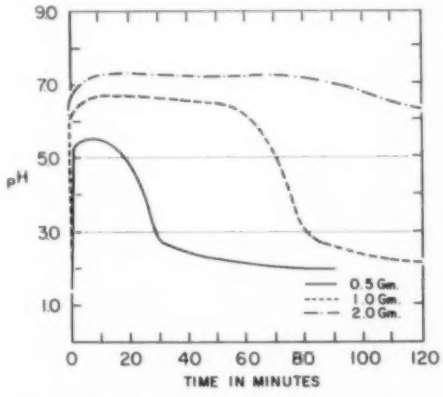


Fig. 15.—Sodium bicarbonate U. S. P., procedure of Holbert, Noble, and Grote modified.

The results, which are plotted in Fig. 17, show that AHMC is somewhat more reactive after four years storage at ambient temperatures than the other two samples.

Comparison with Dry Blend.—To demonstrate the difference in antacid properties between the new AHMC dried gels and a simple dry blend of aluminum hydroxide dried gel U. S. P. and magnesium carbonate U. S. P. having an Al_2O_3:MgO mole ratio of 2:1, the following experiment was performed. Four parts of dried aluminum hydroxide was thoroughly blended with one part of magnesium

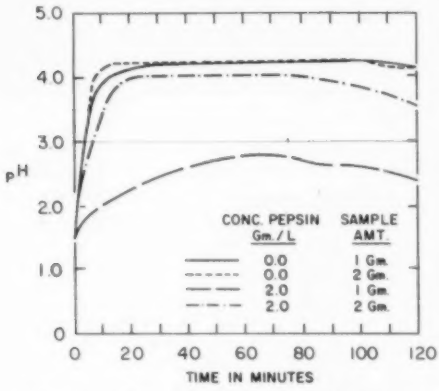


Fig. 16.—Effect of pepsin on antacid activity of dried aluminum hydroxide gel U. S. P., procedure of Holbert, Noble, and Grote modified.

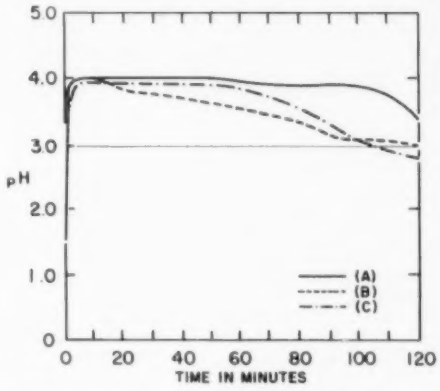


Fig. 17.—Antacid activity of four-year old samples of three aluminum antacids: (*A*) AHMC; (*B*) aluminum dihydroxy sodium carbonate; (*C*) aluminum dihydroxy aminoacetate; procedure of Holbert, Noble, and Grote modified.

carbonate, and the antacid activity of the blend was determined on the basis of 1 Gm. and 2 Gm. The data are shown in Table IX and plotted in Fig. 18.

TABLE IX.—ANTACID ACTIVITY COMPARISON OF AHMC WITH ALUMINUM HYDROXIDE–MAGNESIUM CARBONATE DRY BLEND (4:1)[a]

Time, min.	AHMC		Aluminum Hydroxide–Magnesium Carbonate Dry Blend (Mole ratio—Al_2O_3:MgO—2:1)	
	1 Gm.	2 Gm.	1 Gm.	2 Gm.
0	1.5	1.5	1.5	1.5
1	4.0	4.1	4.5	5.8
2	4.1	4.3	4.5	6.4
3	4.2	4.3	4.6	6.6
4	4.2	4.4	4.7	6.8
5	4.2	4.4	4.7	6.9
10	4.3	4.5	4.8	7.2
20	4.3	4.4	3.8	6.9
30	4.3	4.4	3.1	6.6
40	4.2	4.3	2.8	6.2
50	4.2	4.3	2.7	5.7
60	4.2	4.3	2.6	4.6
70	4.2	4.3	2.5	3.7
80	4.2	4.3	2.4	3.1
90	4.1	4.2	2.4	2.7
100	4.1	4.2	2.3	2.5
110	4.1	4.2	2.3	2.4
120	4.0	4.2	2.3	2.3

[a] Time *versus* pH, procedure of Holbert, Noble, and Grote.

RESULTS

The data in Tables III and IV show that by both reaction velocity tests AHMC dried gels are rapidly reactive with 0.1 *N* HCl at 37.5° and compare favorably with a highly reactive aluminum hydroxide gel U. S. P. (prepared from Reheis F-500 aluminum hydroxide compressed gel). Aluminum dihydroxy sodium carbonate, which is also shown to be rapidly reactive by the Reheis reaction velocity test, contains 306 mg. of sodium oxide for each 500 mg. of aluminum oxide. Aluminum dihydroxy aminoacetate and sodium polyhydroxy aluminum monocarbonate hexitol complex are only slightly less reactive than AHMC.

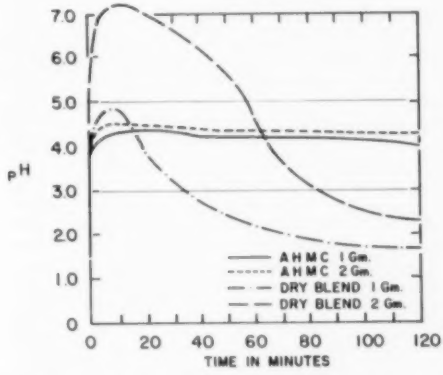


Fig. 18—Comparison of antacid activity of AHMC with aluminum hydroxide-magnesium carbonate dry blend (4:1), procedure of Holbert, Noble, and Grote modified.

The data in Table V, which are plotted in Figs. 3–15, show that both types of AHMC dried gel are very rapid and prolonged in their antacid activity in the pH range of 3 to 5 with artificial gastric juice. In this respect, they compare favorably with the ideal, a reactive liquid aluminum hydroxide gel.

The data plotted in Fig. 17 shows that AHMC retains this high degree of reactivity over a long period of time. Not a single substance tested shows better results than the AHMC dried gels. Table VII shows that the superior antacid characteristics of AHMC is retained in tablets as well. Hinkel, Fisher, and Tainter (25, 26) described a new highly reactive aluminum hydroxide (sodium polyhydroxy aluminum monocarbonate hexitol complex) which, when blended with magnesium hydroxide, forms the basis of an improved antacid tablet. The data for sample 43, Table VII indicates that the antacid characteristics of this new improved tablet are excellent and compare favorably with AHMC powder and tablets.

DISCUSSION

A new combination of two old antacids, aluminum hydroxide and magnesium carbonate, is shown to possess outstanding antacid properties. The principal method employed in this *in vitro* study is a very stringent test. It was Johnson and Duncan who first introduced the periodic replacement of antacid-acid mixture with the fresh gastric acid. They used an equivalent dose, which varied for each product tested, depending upon the acid neutralizing power of the substance. Holbert, Noble, and Grote (28) used a 2-Gm. sample and employed an artificial gastric juice instead of 0.1 *N* HCl. Our modification consisted of (*a*) establishing an exact normality for the artificial gastric juice and (*b*) replacing it at a constant rate. The rate of change of pH with time during the first ten minutes is a function of its reactivity and, hence, the test is unexcelled for measuring promptness of buffering. Since, at the ten-minute interval an excess of antacid is usually present, the pH at ten minutes is a measure of the maximum pH that might be expected. The concentration of acid (pH 1.5–0.0316 *N*) in the artificial gastric juice also makes the test a very stringent one. A substance which reacts well with acid of this strength will react even more rapidly with a stronger acid. The constant replacement of reaction mixture with fresh gastric juice provides a system where the amount of ant-antacid is gradually diminishing while the supply of fresh acid is constant. A more stringent test for an antacid substance cannot be devised. One, of course, may vary the concentration and composition of the gastric juice and obtain a whole family of curves and data which is constant for the new gastric juice. We have used simulated gastric fluid U. S. P., which became official after our investigation was started. This is approximately 0.08 *N* but may vary depending on the concentration of hydrochloric acid used. Our results with this more concentrated gastric juice show that the total time in the pH range of 3 to 5 is less, but that the promptness of reaction and duration of action is similar for AHMC dried gel and liquid aluminum hydroxide gel.

The *in vitro* procedure, as employed, by us is not represented as being a duplication of the behavior of an antacid in the human stomach. It is perhaps the most severe method of comparing antacids that can be devised under conditions similar to those found in the stomach. It is doubtful whether any *in vivo* test so far described is fully adequate to differentiate between a large number of antacids. The final and true test must remain in the hands of competent clinicians.

Nature of Aluminum Hydroxide Gel.—Reactive aluminum hydroxide gel is a very dynamic system whose properties are dependent on the presence of immobilized watershells and the nature and amount of absorbed foreign anions and cations. The loss of protons, which are expelled from the hydration shells as a result of electrostatic attraction between an aluminum ion and an hydroxyl group in a neighboring hydration shell, brings about a polymerization. This structure can be stabilized by substances which help to retain the remaining protons in the hydration shells. Submicroscopic particles of magnesium carbonate is an excellent stabilizer of this structure.

SUMMARY

1. The preparation and properties of two new aluminum hydroxide–magnesium carbonate dried gels have been described.

2. *In vitro* evaluation of the antacid properties of AHMC dried gels by four methods demonstrate that its buffering action is very rapid and prolonged in the optimum pH range of 3 to 5. It compares favorably in this respect with reactive liquid aluminum hydroxide gels.

3. The outstanding antacid properties of AHMC are retained over a long period of time.

4. Of all other antacid chemicals tested in powder form, only aluminum dihydroxy aminoacetate, aluminum dihydroxy sodium carbonate, and sodium polyhydroxy aluminum monocarbonate hexitol complex exhibited prompt and prolonged activity in the pH range of 3 to 5.

5. *In vitro* antacid evaluation data on 30 of the most widely used antacids in liquid and tablet form are included for purposes of comparison.

REFERENCES

(1) Einsel, I. H., Adams, W. L., and Myers, V. C., *Am. J. Digest. Diseases*, **1**, 513(1934).
(2) Woldman, E. E., and Rowland, V. C., *Rev. Gastroenterol.*, **3**, 27(1936).
(3) Emery, E. S., Jr., and Rutherford, R. B., *Am. J. Digest. Diseases*, **5**, 486(1938).
(4) Eads, J. T., *ibid.*, **7**, 32(1940).
(5) Jones, C. R., Jr., *Am. J. Digest. Diseases Nutrition*, **4**, 99(1937).
(6) Connelly, A., and Hollander, F., *Rev. Gastroenterol.*, **9**, 354(1942).
(7) Kyger, E. R., Jr., Hashinger, E. H., and Wilbelmj, E. W., *Am. J. Digest. Diseases*, **6**, 363(1939).
(8) Kunstler, M. B., *Med. Record*, **149**, 268(1939).
(9) Bennett, T. I., and Gill, A. M., *Lancet*, **1**, 500(1939).
(10) Steigmann, F., *Illinois Med. J.*, **76**, 443(1939).
(11) Woldman, E. E., and Polan, C. G., *Am. J. Med. Sci.*, **198**, 155(1939).
(12) Fauley, G. B., Ivy, A. C., Terry, L., and Bradley, W. B., *Am. J. Digest. Diseases*, **5**, 792(1939).
(13) Cornell, A., Hollander, F., and Winkelstein, A., *ibid.*, **9**, 332(1942).
(14) McIntosh, J. F., and Sutherland, C. G., *Can. Med. Assoc. J.*, **42**, 140(1940).
(15) Hardt, L. L., and Brodt, L. P., *Arch. Surg.*, **55**, 584 (1947).
(16) Hardt, L. L., and Steigmann, F., *Am. J. Digest. Diseases*, **17**, 195(1950).
(17) Morrison, S., *Am. J. Gastroenterol.*, **21**, 301(1954).
(18) Collins, E. N., *J. Am. Med. Assoc.*, **127**, 899(1945).
(19) Rossett, N. E., and Flexner, J., *Ann. Internal Med.*, **21**, 119(1944).
(20) Rossett, N. E., and Flexner, J., *ibid.*, **18**, 193(1943).
(21) Johnson, E. H., and Duncan, J., *Quart. J. Pharm. and Pharmacol.*, **18**, 251(1945).
(22) Hammarlund, E. R., and Rising, L. W., THIS JOURNAL, **38**, 586(1949).
(23) Dale, J. K., and Booth, R. E., *ibid.*, **44**, 170(1955).
(24) Gwilt, J. R., Livingston, J. L., and Robertson, A., *J. Pharm. and Pharmacol.*, **10**, 770(1958).
(25) Hinkel, E. T., Jr., Fisher, M. P., and Tainter, M. L., THIS JOURNAL, **48**, 380(1959).
(26) Hinkel, E. T., Jr., Fisher, M. P., and Tainter, M. L., *ibid.*, **48**, 384(1959).
(27) Murphey, R. S., *ibid.*, **41**, 361(1952).
(28) Holbert, J. M., Noble, N., and Grote, I. W., *ibid.*, **37**, 292(1948).
(29) Holbert, J. M., Noble, N., and Grote, I. W., *ibid.*, **36**, 149(1947).
(30) Schenk, J., *Schweiz. med. Wochschr.*, **84**, 1418(1954).
(31) Mutch, N., *Quart. J. Pharm. and Pharmacol.*, **19** 490(1946).

Preparation and Properties of New Gastric Antacids II*

Aluminum Hydroxide–Protein Dried Gels

By STEWART M. BEEKMAN and CARL H. VOGEL

Seven new antacids are described. Each is a combination of aluminum hydroxide and protein in dry form. The *in vitro* technique (based on the procedure of Holbert, Noble, and Grote) described in the first paper of this series is used to evaluate the antacid properties of the new dried gels. They are shown to be prompt in raising the pH to about 4 and prolonged in maintaining the pH in the optimum range of 3 to 5. All are palatable and free from chalky taste. The compositions include combinations of aluminum hydroxide with nonfat milk, milk protein concentrate, whole milk, egg albumin, lactalbumin, gelatin, and a soya milk product.

In the first paper (1) in this series of studies, two new aluminum hydroxide–magnesium carbonate dried gels (AHMC) were described and their excellent antacid characteristics were determined by a modified *in vitro* procedure of Holbert, Noble, and Grote (2). A specially designed apparatus for evaluating antacid activity was also described.

The purpose of this paper is to describe several new aluminum hydroxide–protein dried gels and to present *in vitro* antacid evaluation data using the same procedure and apparatus.

Several investigators have endeavored to prepare effective antacids by combining proteins with aluminum. Lipschitz (3) proposed a blend of 29.2 parts of aluminum hydroxide dried gel with 400 parts of calcium caseinate and 100 parts of calcium carbonate. Horlicks (4) mixed 10 parts of aluminum hydroxide dried gel with 38 parts of malted milk. Paterson (5) reacted a soluble protein with aluminum ions or gel particles under conditions to precipitate an aluminum proteinate. Our evaluation of these products shows that they do not meet the requirements for a successful, modern antacid in dry form.

EXPERIMENTAL

The antacids which are the subject of this study were all prepared by intimately contacting an aqueous solution of soluble, edible protein with a highly reactive basic aluminum carbonate gel at a pH sufficiently high to prevent precipitation, followed by desiccation and reduction to finely divided form. The proteins used were nonfat milk, milk protein concentrates, soluble lactalbumin, whole milk, egg albumin, gelatin, and a milk substitute containing soya protein. The products were white, odorless dried gels having a pleasant, nonchalky, and bland taste. In Table I the composition of several aluminum hydroxide–protein dried gels are shown. In Table II the analyses of the various samples as well as their acid consuming capacities can be found.

Table I.—Composition of Various Aluminum Hydroxide–Protein Dried Gels

Designation	Composition
ALUMIL[a] F-21	$Al(OH)_3$[b]–Nonfat milk solids (2:1)
AHEA F-41	$Al(OH)_3$–Egg albumin (4:1)
AHLA F-41	$Al(OH)_3$–Lactalbumin (4:1)
AHGEL F-41	$Al(OH)_3$–Gelatin (4:1)
AHMPC F-41	$Al(OH)_3$–Milk protein concentrate (4:1)
AHS F-41	$Al(OH)_3$–Soya milk (4:1)
AHWM F-11	$Al(OH)_3$–Whole milk solids (1:1)

[a] Registered trademark, Reheis Co., Inc.
[b] $Al(OH)_3$ as Al_2O_3.

Antacid Activity.—The antacid activity of the various samples of aluminum hydroxide–protein dried gels was determined by the modified procedure of Holbert, Noble, and Grote (2) on the basis of 1-2-Gm. doses. In addition, the antacid activity of 10-grain aluminum hydroxide–non-fat milk tablets (ALUMIL F-21) was determined on the basis of one and two tablets. The results of these determinations are shown in Table III and Figs. 1–8.

Effect of Nonfat Milk Solids on Antacid Activity of Aluminum Hydroxide Dried Gel.—In order to determine the effect of nonfat milk solids on the antacid activity of aluminum hydroxide dried gel, two parts of the latter were blended thoroughly with one part of nonfat milk solids (Carnation Instant). The antacid activity of a 3-Gm. sample was determined by the modified procedure of Holbert, Noble, and Grote and compared with a 2-Gm. sample of dried aluminum hydroxide gel. The data which are plotted in Fig. 9 show that nonfat milk solids have an adverse effect on the antacid properties of aluminum hydroxide dried gel. Similar results were obtained with whole milk solids (Klim.). A 2-Gm. sample of nonfat milk solids (Carnation Instant), similarly tested, raised the pH slowly to 2.4 and gradually declined to less than 2.0. A 1-Gm. mixture of 38 parts malted milk with 10 parts aluminum hydroxide dried gel (4) failed to raise the pH of gastric juice above 2.0 during the entire two-hour testing period.

One gram of a Lipschitz (3) mixture, previously described, raised the pH fairly rapidly to 3.6, and

* Received September 2, 1959, from the Research Laboratory, Reheis Co., Inc., Berkeley Heights, N. J.
Presented to the Scientific Section, A. Ph. A., Cincinnati meeting, August 1959.

TABLE II.—ANALYSES OF VARIOUS ALUMINUM HYDROXIDE–PROTEIN DRIED GELS

Designation	Al_2O_3, %	Protein, %	Nonfat Milk Solids, %	CO_2, %	Water, Karl Fischer, %	pH	Acid Consuming Cap.[a]
ALUMIL F-21	38.0	7.0	19.0	12.1	18.8	9.0	220
AHEA F-41	46.1	11.4	..	9.5	16.6	9.1	257
AHLA F-41	46.0	9.2	..	12.5	22.1	9.0	256
AHGEL F-41	42.5	10.5	..	11.2	17.0	9.1	234
AHMPC F-41	42.2	6.8	..	12.7	18.6	9.1	231
AHS F-41	42.4	2.9	..	15.0	22.4	9.3	232
AHWM F-11	31.7	8.7	..	8.9	20.1	9.1	179

[a] Ml. 0.1 N HCl per Gm.

TABLE III.—ANTACID CHARACTERISTICS OF VARIOUS ALUMINUM HYDROXIDE–PROTEIN DRIED GELS[a]

Time, min	ALUMIL F-21, 1 Gm.	ALUMIL F-21, 2 Gm.	AHEA F-41, 1 Gm.	AHEA F-41, 2 Gm.	AHLA F-41, 1 Gm.	AHLA F-41, 2 Gm.	AHGEL F-41, 1 Gm.	AHGEL F-41, 2 Gm.	AHMPC F-41, 1 Gm.	AHMPC F-41, 2 Gm.	AHS F-41, 1 Gm.	AHS F-41, 2 Gm.	AHWM F-11, 1 Gm.	AHWM F-11, 2 Gm.	ALUMIL Tablets, 1 Tab.	ALUMIL Tablets, 2 Tab.
0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
1	3.7	4.1	2.5	4.1	2.5	4.1	2.8	4.1	3.6	4.2	2.5	4.2	3.3	4.1	2.2	2.8
2	4.1	4.2	3.2	4.2	3.0	4.2	3.8	4.2	4.1	4.3	3.2	4.3	3.8	4.2	2.6	3.5
3	4.2	4.2	3.9	4.2	3.5	4.3	4.0	4.2	4.2	4.3	3.8	4.3	4.0	4.2	3.1	3.9
4	4.2	4.2	4.1	4.2	3.9	4.3	4.0	4.2	4.2	4.3	4.0	4.3	4.0	4.2	3.5	4.0
5	4.2	4.2	4.1	4.2	4.0	4.3	4.0	4.2	4.2	4.4	4.1	4.4	4.1	4.2	3.7	4.1
10	4.3	4.3	4.3	4.3	4.2	4.4	4.1	4.2	4.2	4.4	4.1	4.4	4.2	4.2	4.1	4.2
20	4.3	4.3	4.2	4.3	4.2	4.4	4.1	4.2	4.2	4.4	4.1	4.4	4.1	4.2	4.1	4.2
30	4.3	4.2	4.2	4.3	4.2	4.4	4.1	4.2	4.2	4.4	4.1	4.3	4.0	4.1	4.1	4.2
40	4.3	4.2	4.2	4.3	4.2	4.4	4.1	4.2	4.2	4.4	4.1	4.3	4.0	4.1	4.1	4.2
50	4.3	4.2	4.2	4.2	4.2	4.4	4.1	4.1	4.2	4.4	4.1	4.3	4.0	4.1	4.0	4.1
60	4.3	4.2	4.2	4.2	4.2	4.4	4.1	4.1	4.2	4.3	4.1	4.3	3.9	4.1	4.0	4.1
70	4.3	4.2	4.2	4.2	4.1	4.3	4.0	4.1	4.2	4.3	4.0	4.3	3.8	4.0	3.9	4.1
80	4.2	4.2	4.1	4.2	4.1	4.3	4.0	4.1	4.1	4.3	4.0	4.3	3.8	4.0	3.8	4.0
90	4.2	4.2	4.1	4.2	4.1	4.3	4.0	4.1	4.1	4.3	3.9	4.3	3.6	4.0	3.6	4.0
100	4.2	4.2	4.1	4.2	4.0	4.2	3.9	4.1	4.0	4.3	3.7	4.3	3.4	3.9	3.4	3.9
110	4.1	4.2	4.1	4.2	4.0	4.2	3.8	4.1	4.0	4.2	3.3	4.3	3.2	3.9	3.2	3.7
120	4.1	4.2	4.0	4.2	3.9	4.2	3.7	4.1	3.7	4.2	2.9	4.2	2.9	3.8	2.9	3.6
130	4.0	4.1	4.0	4.2	3.8	4.2	3.3	4.0	3.3	4.2	..	4.2	..	3.8	..	3.3
140	3.8	4.1	3.8	4.1	3.5	4.2	3.0	4.0	3.0	4.1	..	4.2	..	3.6	..	..
150	3.5	4.0	3.7	4.1	..	4.2	..	4.0	2.7	4.0	..	4.1	..	3.4	..	..
160	3.2	4.0	3.3	4.1	..	4.1	..	3.9	2.6	4.0	..	4.1	..	3.2	..	..
170	3.0	3.7	3.0	4.1	..	4.0	..	3.9	2.4	3.9	..	3.9	..	2.9	..	..
180	..	3.5	..	4.0	..	3.8	..	3.8	2.3	3.4	..	3.6	..	2.7	..	..
190	..	3.3	..	3.9	..	3.3	..	3.6	..	3.0	..	3.1	..	..	..	..
200	..	2.9	..	3.7	..	3.0	..	3.3	..	2.8	..	2.8	..	..	..	..
210	..	..	..	3.5	..	2.8	..	3.0	..	2.6	..	2.6	..	..	..	..

[a] Time BETWEEN pH at various dose levels, procedure of Holbert, Noble, and Grote modified.

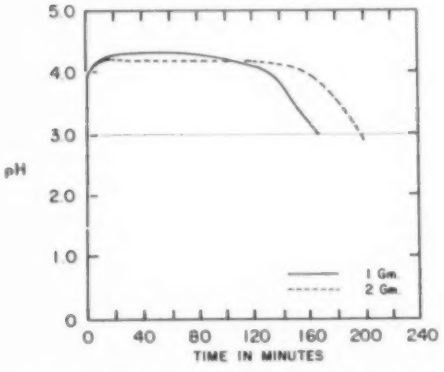


Fig. 1.—Aluminum hydroxide-nonfat milk dried gel, ALUMIL F-21, procedure of Holbert, Noble, and Grote modified.

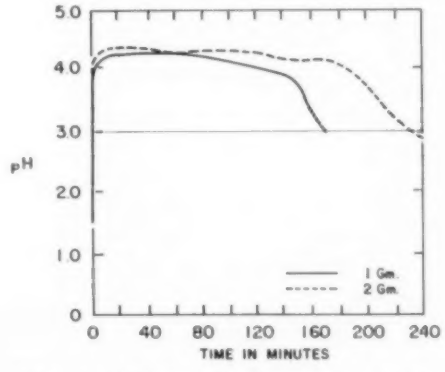


Fig. 2.—Aluminum hydroxide-egg albumin dried gel, AHEA F-41, procedure of Holbert, Noble, and Grote modified.

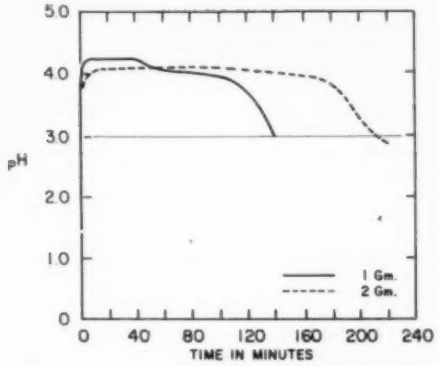


Fig. 3.—Aluminum hydroxide-gelatin dried gel, AHGEL F-41, procedure of Holbert, Noble, and Grote modified.

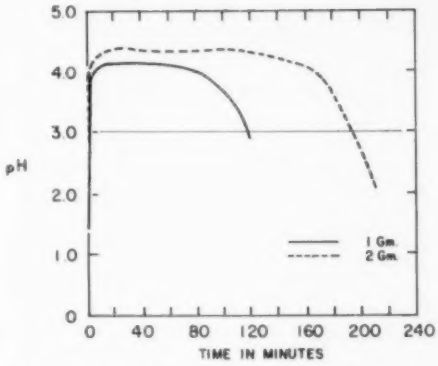


Fig. 6.—Aluminum hydroxide-soya dried gel, AHS F-41, procedure of Holbert, Noble, and Grote modified.

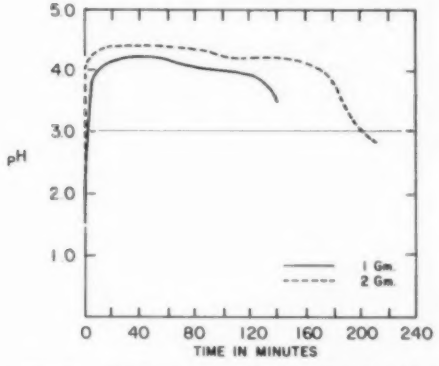


Fig. 4.—Aluminum hydroxide-lactalbumin dried gel, AH-LA F-41, procedure of Holbert, Noble, and Grote modified.

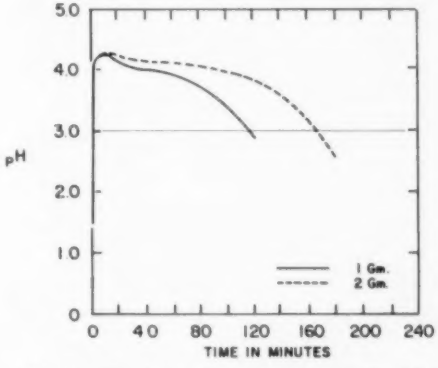


Fig. 7.—Aluminum hydroxide-whole milk dried gel, AHWM F-11, procedure of Holbert, Noble, and Grote modified.

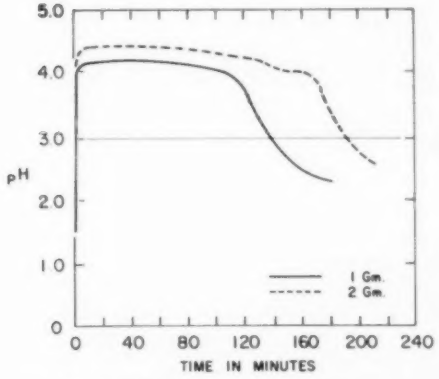


Fig. 5.—Aluminum hydroxide-milk protein concentrate dried gel, AHMPC F-41, procedure of Holbert, Noble, and Grote modified.

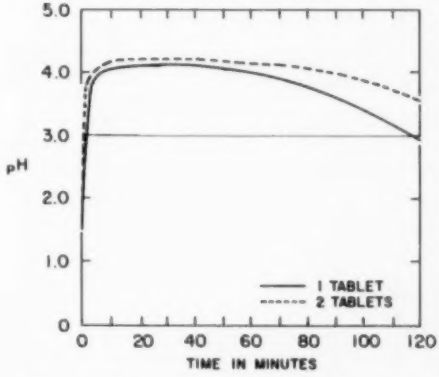


Fig. 8.—Alumil F-21 tablets, 660 mg. active ingredient, procedure of Holbert, Noble, and Grote modified.

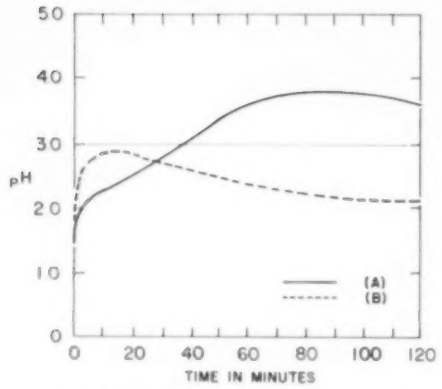


Fig. 9.—Effect of dry nonfat milk solids on antacid activity of aluminum hydroxide dried gel U. S. P.: (A) Aluminum hydroxide dried gel, 2 Gm., (B) Aluminum hydroxide dried gel, 2 Gm. plus 1 Gm. nonfat milk solids; procedure of Holbert, Noble, and Grote modified.

thereafter, slowly declined to 3.0 in twenty minutes and 2.0 after one hundred minutes. The antacid characteristics of aluminum proteinate (5) are shown in Fig. 9 and in Table V of a previous paper (1).

RESULTS

The data on the antacid activity of various aluminum hydroxide–protein dried gels, which are shown in Table III and Figs. 1–8, indicate they are prompt in raising the pH to 3.5 and very prolonged in maintaining the pH in the optimum range of 3 to 5. The maximum pH reached with a large excess of antacid was about 4.4. One-gram samples maintained the pH in the optimum range for well over two hours and 2-Gm. samples for well over three hours. This is in sharp contrast with the data shown in Fig. 9, which show that nonfat milk solids have an adverse effect on the antacid activity of aluminum hydroxide dried gel. The data plotted in Fig. 8 show that the prompt and prolonged antacid activity of ALUMIL F-21 is retained in tablets as well.

DISCUSSION

The antacid properties of aluminum hydroxide–protein dried gels, as determined by the modified *in vitro* technique of Holbert, Noble, and Grote, have been shown to compare favorably with reactive liquid aluminum hydroxide gel. The results obtained with aluminum hydroxide–nonfat milk (ALUMIL), which is a most economical source of protein, were comparable with others from the point of view of promptness and long lasting buffering power in the optimum pH range. Tablets made from ALUMIL were characterized by a smooth, bland, nonchalky taste and unexcelled sucking quality. A vanillin flavor gave a pleasant taste and aftertaste. Favorable comments were elicited from individuals who dislike "chalky" or highly flavored tablets.

The prolonged buffering quality of ALUMIL suggests its use for night-time therapy of ulcer patients. Other antacid chemicals, such as magnesium hydroxide, can be most effectively incorporated by blending with the wet gel before drying.

SUMMARY

1. The preparation and properties of seven new aluminum hydroxide–protein dried gels were described.

2. Each was shown to exhibit promptness in buffering artificial gastric juice and prolonged antacid action in the optimum pH range of 3 to 5 when evaluated by the modified *in vitro* procedure of Holbert, Noble, and Grote.

3. The antacid properties were shown to compare favorably with reactive liquid aluminum hydroxide gel.

4. The antacid activity of ALUMIL F-21 (aluminum hydroxide–nonfat milk dried gel) tablets was shown to be prompt and prolonged.

5. The aesthetic qualities of aluminum hydroxide–nonfat milk dried gel (ALUMIL) such as the bland, nonchalky, pleasant taste characteristics as well as the smooth, nongritty, sucking qualities which it imparts to tablets have been stressed.

REFERENCES

(1) Beekman, S. M., THIS JOURNAL, **49**, 191(1960).
(2) Holbert, J. M., Noble, N., and Grote, I. W., *ibid.*, **37**, 292(1948).
(3) U. S. pat. 2,362,386.
(4) British pat. 715,874.
(5) U. S. pat. 2,721,861.

The Effects of Certain Psychopharmacologic Drugs on Conditioning in the Rat I*

Avoidance-Escape Conditioning

By V. D. LYNCH†, M. D. ACETO‡, and R. K. THOMS

This study compares the effects on conditioning of well-known classes of tranquilizers both with themselves and with other centrally active drugs. On the basis of certain chemical structure relationships, 2-ethyl-2-(3-methyl)-butylmalondiamide, 2-ethyl-2-(3-methyl)-butylmalonic acid diethylester, and ethyl-2,4-toluene dicarbamate were included for study. The results which were analyzed statistically by means of an analysis of variance technique and *t* tests demonstrated significant differences between drugs and between drugs and controls from both dosage and trial aspects. Because of the nature of the test, these differences cannot be ascribed only to an interference with normal motor activity.

WHILE MANY PROCEDURES have been used for the assessment of tranquilizers, the experimental approach has logically focused on the effects of these drugs on animal behavior (1, 2). In an attempt to contribute somewhat to a further understanding of such phenomena, the following study was carried out, keeping in mind the threefold objectives: (*a*) to determine whether quantitative relationships could be determined not only between drugs but also between different dosage levels of the same drugs; (*b*) to compare the effects of different chemical classes of drugs; and (*c*) to determine whether or not this procedure could be used to evaluate new or untested chemical compounds.

In the study of centrally active drugs on animal behavior, Cook and Weidley (3) utilized a procedure in which a rat was conditioned to climb a pole to escape an electric shock which was delivered simultaneously with a warning tone. After a number of such presentations of shock and tone together, the animal would develop a response termed avoidance-escape by the authors and it would respond to the sound alone.

In the present study, the same procedure was utilized but modified to the extent that a time interval was introduced between the warning tone and the electric shock. In addition, other parameters were extended with regard to the time of administration of drugs. It was also felt that the administration of a drug to an unconditioned animal rather than to one already conditioned would more fully elucidate the effects of the selected drugs.

EXPERIMENTAL PROCEDURES

Materials.—The drugs and dosage levels are listed in Table I. Except for the diamide[1] and dicarbamate (4) which were synthesized in our laboratories, all the other drugs were obtained commercially or supplied by the manufacturers.[2] Distilled water was used as the solvent for the relatively water-soluble drugs. Methocel (0.5%) was used as the suspending agent for the poorly water-soluble drugs. In all cases, the drug was either dissolved or suspended so that the desired dose per Kg. of body weight of the rat was contained in a final volume of 1 cc. of water. When not in use, the solutions were stored in a refrigerator.

Animals.—The animals used in this study were descendants of a Wistar strain originally obtained from the Charles River Breeding Laboratories. Adult rats were kept in colonies of approximately 25 to a standard size rat cage. Separate cages were maintained for both sexes. These animals were allowed Purina laboratory chow for food and water *ad libitum*. Once selected for use in the avoidance-escape procedure, the animals were individually housed in rectangular mouse boxes, and food and water allowed *ad libitum*.

Conditioning Apparatus.—The apparatus consisted of a cage (both wooden and glass containers were used in these experiments) fitted with a cover to which a wooden rod was attached by means of a flat metal spring. This rod protruded through the center of the cover and extended down almost to the floor of the cage. The weight of an animal on this

* Received May 26, 1959, from the School of Pharmacy, University of Connecticut, Storrs.
Abstracted in part from dissertations submitted to the Graduate School by Vincent D. Lynch and Mario D. Aceto in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June 1959.
† Present address: College of Pharmacy, St. John's University, Jamaica 32, N. Y.
‡ Present address: School of Pharmacy, University of Pittsburgh, Pittsburgh 13, Pa.
The authors wish to thank Dr. David Zeaman of the Psychology Department of the University of Connecticut and Dr. Robert Orlando of the Institute for Child Development of the University of Washington for their valuable assistance in developing the psychological procedures and the statistical analysis of the data.

[1] The synthesis and pharmacological data on this compound to be published.
[2] The following drugs were supplied gratus from the firms listed: 2-ethyl-2-(3-methyl)-butylmalonic acid diethyl ester from Eli Lilly and Co.; Equanil (meprobamate) from Wyeth Laboratories; Thorazine hydrochloride from Smith Kline and French Laboratories; Atarax hydrochloride from J. B. Roerig and Co. (Div. of Charles Pfizer & Co., Inc.); Serpasil phosphate (lyophilized crystals) from Ciba Pharmaceutical Products Inc.

TABLE I.—DRUGS AND DOSES USED IN AVOIDANCE-ESCAPE CONDITIONING TECHNIQUE[a]

Drug	Lot and Control No.	Low, mg./Kg.	Medium, mg./Kg.	High, mg./Kg.
Reserpine phosphate	Ciba E 4961	0.5	1.0	2.0
Chlorpromazine hydrochloride	SKF Cu 7664	0.5	2.0	4.0
Meprobamate	Wyeth L 3286-1	10.0	50.0	100.0
Hydroxyzine hydrochloride	Pfizer 71407	5.0	25.0	35.0
d-Amphetamine sulfate	Lack DA 675	0.1	1.0	5.0
Amobarbital	Eli Lilly AX-9643-F	5.0	10.0	20.0
Ethyl-2,4-toluene dicarbamate	....	50.0	75.0	90.0
2-Ethyl-2-(3-methyl)-butylmalondiamide	....	10.0	50.0	100.0
2-Ethyl-2-(3-methyl)-butylmalonic acid diethyl ester	Eli Lilly 11039	50.0	100.0	200.0
Control	....	..	..	..

[a] All drugs given by i. p. injection.

pole would cause the pole to descend, thereby engaging electrical contacts at the top of the pole which immediately terminated the warning sound and/or electric shock. The cage floor was constructed as a grid of copper rods spaced approximately one-half inch apart. The grid floor could be electrically charged by means of a stimulator. The stimulator (Electronic Stimulator, Grass Instrument Co.) frequency was set and maintained at five per second, delay at five milliseconds, duration at thirty milliseconds, and voltage at 100 volts d. c. A resistor was so set that three milliamperes current was delivered to the grid.

The apparatus was constructed so that approximately every five minutes it would initiate a new cycle which began by the sounding of a low tone buzzer for approximately sixteen seconds followed by the delivery of an electric shock and continued sounding of the buzzer (with concurrent shock) until either the animal jumped upon the pole or, as was found necessary for certain drugs during the first hourly session, until the animal became cyanotic. If the animal became cyanotic, the investigator manually depressed the protruding end of the pole. Either manual depression of the pole or the presence of the animal upon the pole served to terminate simultaneously the buzzer tone and electric shock. The apparatus would automatically reset itself and after approximately five minutes initiate a new cycle unless the rat was still on the pole, in which case another cycle would begin approximately five minutes after the animal left the pole. A kymograph (Phipps and Bird, model V H.) separately recorded duration of the tone and shock by means of ink-fed pens attached to signal magnets.

The animal was introduced into the apparatus by raising the removable roof. Two of these devices were used and each apparatus was housed in a separate room in an exhaust hood.

Procedure.—In each of the apparatuses, five rats were used to form a group for each dose level investigated for each drug. Once a group was assigned to an apparatus, it was used only in the apparatus assigned. The similar results obtained for each dose level for each drug from each apparatus were then combined to permit the best possible statistical analysis. Experimentally naïve animals of both sexes of a weight range from 150 to 250 Gm. were used. Aside from the control groups which did not receive any injections, all other groups were injected intraperitoneally one hour prior to each of the five consecutive daily admissions into the apparatus. Reserpine was administered two hours prior and meprobamate one-half hour prior to admission of the animal into the apparatus. Due to the cumulative effects of reserpine, it was found necessary to administer this drug and conduct the conditioning session every other day for a total of three sessions.

After injecting an animal with the selected drug it was replaced in its assigned cage and remained there until ready for introduction into the apparatus. The animal was removed from the cage and placed in tap water of sufficient depth to allow wetting of its limbs and then admitted into the apparatus. After the first few shocks the animal learned to find the pole and responded in time according to the effects of the drug. After a conditioning session, each rat was returned to its assigned cage.

RESULTS

In the evaluation of the data, the original measurements on the kymograph record were made in millimeters and then converted to response latency in seconds. Response latency may be described as the time interval which elapses between the onset of the warning tone and response of the animal, in this case, climbing the pole to avoid an oncoming shock. Each animal received an average of ten trials in each daily session, and because the results of these trials showed skewness, the measurement for each trial was converted to its reciprocal.

The average of these reciprocals ($\times$ 100) for each animal on each day was entered into a table and analyzed statistically by means of a split-plot analysis of variance technique (5). The results of this technique are based on the measurements of approximately 15,000 individual trials and are given in Table II.

Using the *t* test[3] as defined by Edwards (6), a further statistical evaluation of the various drug to drug and drug to control comparisons was made. Those results are listed in Tables III, IV, and V. Figures 1 through 9 illustrate the trial-latency curves for the different drugs and doses investigated. Table VI provides a summary and tabulation of the drugs and dose levels at which significant results were obtained.

[3]

TABLE OF *t* VALUES (6)

	P =	
df	0.05	0.01
18	2.101	2.878

TABLE II.—SPLIT-PLOT ANALYSIS OF VARIANCE OF THE EFFECTS OF NINE DRUGS ON THE ACQUISITION OF CONDITIONING OF WHITE RATS IN AN AVOIDANCE-ESCAPE SITUATION USING RESPONSE LATENCY AS THE MEASURE[a]

Source of Variation	Sum of Squares	df	Mean Square	F	F 1% (Fisher's Tables)
Main plot					
A-Drugs	171,680.41	8	21,460.05	15.39	2.60
B-Levels	56,580.05	2	28,290.02	20.09	4.71
A × B Interaction	118,500.07	16	7,406.25	5.31	2.09
Main plot error	338,667.23	243	1,393.69	..	..
Subplot					
T-Trials	86,537.71	4	21,634.42	56.38	3.32
A × T Interaction	27,030.02	32	844.69	2.20	1.69
B × T Interaction	5,023.20	8	627.90	1.64	2.51
A × B × T Interaction	52,481.45	64	820.02	2.14	1.54
Subplot error	337,003.60	972	383.75	..	..
Total	1,193,503.74	1,349	...	..	..

[a] Each drug tested at three different dosage levels.

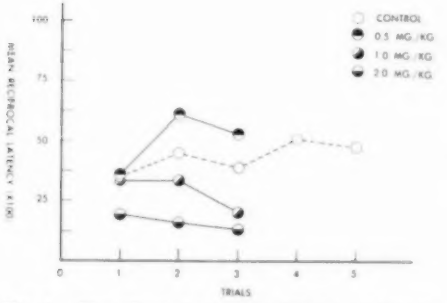


Fig. 1.—The effect of reserpine PO_4 on avoidance-escape.

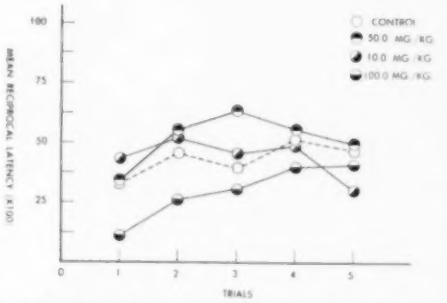


Fig. 3.—The effect of meprobamate on avoidance-escape.

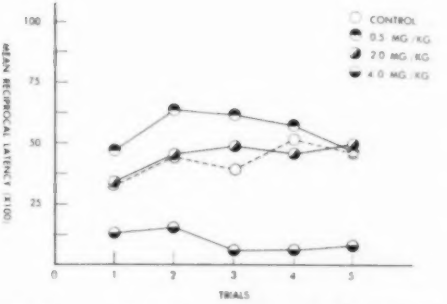


Fig. 2.—The effect of chlorpromazine HCl on avoidance-escape.

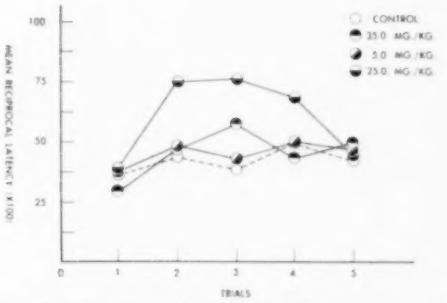


Fig. 4.—The effect of hydroxyzine HCl on avoidance-escape.

DISCUSSION AND SUMMARY

The F values obtained by means of a split-plot analysis of variance of the data indicate that many significant drug to drug differences exist considered from both dosage and trial aspects. The *t* tests derived from these data lend further support and indicate the specific drug to drug significant differences and the drug to control significant differences.

On the assumption of the validity of the significance of the F ratios for both the trial and trial-drug interactions, an inspection of the different curves in Figs. 1 through 9 shows that the differences generally arise on the second trial, and that generally these differences are maintained throughout the remaining three trials.

Reserpine was effective in interfering with the avoidance-escape response significantly in a dose of one to two mg./Kg. The data, as presented, does

TABLE III.—TABLE OF *t* VALUES DERIVED FROM VARIOUS DRUG TO DRUG AND DRUG TO CONTROL COMPARISONS, LOW DOSAGE LEVEL

	Drug	1	2	3	4	5	6	7	8	9	10
1	Reserpine phosphate		1.80	1.34	0.88	0.17	0.13	1.70	2.78	1.49	1.08
2	Chlorpromazine hydrochloride			1.29	0.89	0.28	0.77	1.24	1.93	0.96	1.04
3	Meprobamate				0.64	1.48	2.48	3.15	5.09	3.41	0.20
4	Hydroxyzine hydrochloride					1.05	2.19	2.85	4.04	3.14	0.36
5	*d*-Amphetamine sulfate						0.93	1.48	2.44	1.22	1.23
6	Amobarbital							0.57	1.35	0.09	2.22
7	Ethyl-2,4-toluene dicarbamate										2.78
8	2-Ethyl-2-(3-methyl)-butyl malondiamide									1.89	4.23
9	2-Ethyl-2-(3-methyl)-butyl malonic acid diethyl ester										2.86
10	Control										

df = 18

TABLE IV.—TABLE OF *t* VALUES DERIVED FROM VARIOUS DRUG TO DRUG AND DRUG TO CONTROL COMPARISONS, MEDIUM DOSAGE LEVEL

	Drug	1	2	3	4	5	6	7	8	9	10
1	Reserpine phosphate		2.64	3.42	6.60	6.68	6.07	6.23	2.17	5.66	3.17
2	Chlorpromazine hydrochloride			0.68	2.05	2.72	2.20	2.28	0.81	1.70	0.11
3	Meprobamate				1.20	1.94	1.21	1.50	1.57	0.89	0.89
4	Hydroxyzine hydrochloride					1.09	0.09	0.47	3.58	0.35	2.64
5	*d*-Amphetamine sulfate						0.95	0.59	4.12	1.33	3.32
6	Amobarbital							0.36	0.34	0.40	2.61
7	Ethyl-2,4-toluene dicarbamate										2.83
8	2-Ethyl-2-(3-methyl)-butyl malondiamide									3.02	0.83
9	2-Ethyl-2-(3-methyl)-butyl malonic acid diethyl ester										2.17
10	Control										

df = 18

TABLE V.—TABLE OF *t* VALUES DERIVED FROM VARIOUS DRUG TO DRUG AND DRUG TO CONTROL COMPARISONS, HIGH DOSAGE LEVEL

	Drug	1	2	3	4	5	6	7	8	9	10
1	Reserpine phosphate		0.76	3.76	4.55	4.70	4.78	10.96	7.24	5.59	4.51
2	Chlorpromazine hydrochloride			5.73	5.84	5.63	6.96	14.29	9.08	7.79	5.75
3	Meprobamate				2.09	2.48	1.74	8.14	4.45	2.82	1.56
4	Hydroxyzine hydrochloride					0.32	0.94	3.47	1.37	0.03	0.68
5	*d*-Amphetamine sulfate						1.28	2.91	0.95	0.34	1.00
6	Amobarbital							5.93	2.87	1.24	0.24
7	Ethyl-2,4-toluene dicarbamate										4.92
8	2-Ethyl-2-(3-methyl)-butyl malondiamide									1.66	2.34
9	2-Ethyl-2-(3-methyl)-butyl malonic acid diethyl ester										0.89
10	Control										

df = 18

TABLE VI.—DRUGS SHOWING A SIGNIFICANT EFFECT ON RESPONSE TIME WHEN COMPARED TO CONTROL (P = 0.05)

Drug	Effect	Dose, mg./Kg.	No response effect at following doses, mg./Kg.
Reserpine phosphate	Inhibited response	1.0, 2.0	0.5
Chlorpromazine hydrochloride	Inhibited response	4.0	0.5, 2.0
Hydroxyzine hydrochloride	Facilitated response	25.0	5.0, 35.0
d-Amphetamine sulfate	Facilitated response	1.0	0.1, 5.0
Amobarbital	Facilitated response	5.0, 10.0	20.0
Ethyl-2,4-toluene dicarbamate	Facilitated response	50.0, 75.0, 90.0	.....
2-Ethyl-2-(3-methyl)-butyl malondiamide	Facilitated response	10.0, 100.0	50.0
2-Ethyl-2-(3-methyl)-butyl malonic acid diethyl ester	Facilitated response	50.0, 100.0	200.0
Meprobamate	.....	.....	10.0, 50.0, 100.0

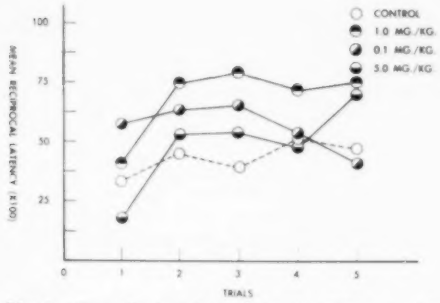


Fig. 5.—The effect of *d*-amphetamine SO_4 on avoidance-escape.

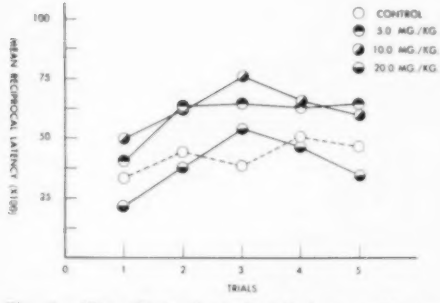


Fig. 6.—The effect of amobarbital on avoidance-escape.

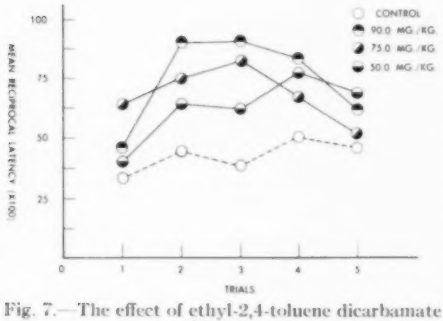


Fig. 7.—The effect of ethyl-2,4-toluene dicarbamate on avoidance-escape.

not justify a conclusion as to whether this drug interfered with the acquisition of conditioning, or whether it prevented the animal from responding. Diarrhea, and ptosis of the eyelids, in addition to sedation, were obvious in all animals receiving this drug by the third trial (in this case five days after the first trial). The highest dose of reserpine was the first to manifest these results. The activity of reserpine phosphate was found to be cumulative.

Chlorpromazine hydrochloride was found to have a significant effect on conditioning in a four mg./Kg. dose. The curve for this drug indicates that at this dose chlorpromazine appears to interfere with the

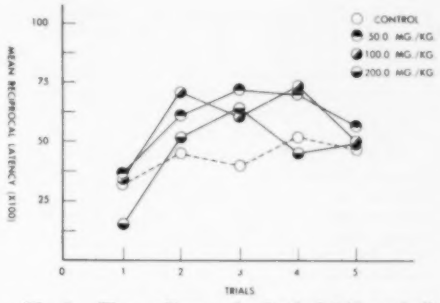


Fig. 8.—The effect of 2-ethyl-2-(3-methyl)-butyl malonic acid diethyl ester on avoidance-escape.

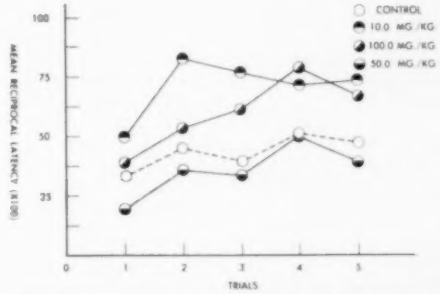


Fig. 9.—The effect of 2-ethyl-2-(3-methyl)-butylmalondiamide on avoidance-escape.

avoidance response to a greater extent than with the escape component. However, the response at this dose level is not significantly different from that for reserpine at the highest dose of the latter.

According to the *t* tests, meprobamate does not interfere significantly with conditioning at any dose level. An examination of the response curves indicates an increase in response latency for the highest dose, especially on trial days two and four. This might be consistent with the observation of pronounced flaccid paralysis in all the rats studied at this dose.

The effects of hydroxyzine hydrochloride on avoidance-escape conditioning as reflected by the *t* tests indicate a decrease in response latency at a dose of 25 mg./Kg., whereas no significant effect occurred at a dose of 35 mg./Kg. It is proposed that the highest dose used was beginning to interfere with its own activity at this point, since doses higher than 35 mg./Kg. could not be investigated because such doses caused pronounced convulsive seizures.

It is possible that amphetamine sulfate followed the same pattern of activity since similar results were obtained with this drug, i. e., the median dose facilitating the response and the stimulatory effects of the highest dose interfering with this facilitation.

On the other hand, amobarbital significantly decreased response latency at the low and median dose levels when compared to the controls. This would be consistent with the observation that barbiturates

frequently cause some stimulation prior to depression. This is further borne out by the fact that the animals receiving the highest dose of this drug showed signs of sedation and that depression on the fifth trial day was markedly present when compared to the control group.

Ethyl-2,4-toluene dicarbamate presents an anomaly of effects which are not explainable on the basis of present experimental evidence. In preliminary tests, this compound demonstrated strongly sedative properties. This does not agree with the facilitation of the conditioning response as shown by the *t* tests. Because of the apparently contradictory results achieved with this compound, a more exhaustive pharmacological study of its properties is being undertaken.

The effect of 2-ethyl-2-(3-methyl)-butylmalondiamide on avoidance-escape conditioning, as indicated by *t* tests and inspection of the curves in Fig. 8, shows, that at the lowest dose a significant decrease in latency of response resulted. It was also found that at this dose no significant difference between 2-ethyl-2-(3-methyl)-butyl malonic acid diethylester and 2-ethyl-2-(3-methyl)-butylmalondiamide was apparent. Also, no significant difference between 2-ethyl-2-(3-methyl)-butylmalondiamide and amobarbital was evident at the three doses used. Significant differences were obtained between meprobamate and 2-ethyl-2-(3-methyl)-butylmalondiamide at the high and low dose levels. However, other tests (to be published) indicated that the effects of the diamide were not unlike certain effects produced by meprobamate and amobarbital.

In general, 2-ethyl-2-(3-methyl)-butylmalonic acid diethylester showed effects similar to those produced by the corresponding diamide. In view of the fact that higher doses of the diethylester demonstrated less pronounced effects when compared to the diamide, it is felt that the former is less effective.

REFERENCES

(1) Kety, S. S., *Ann. N. Y. Acad. Sci.*, **66**, 3(1957).
(2) Garattini, S., and Ghetti, V., "Psychotropic Drugs," Elsevier Publishing Co., New York, N. Y., 1957.
(3) Cook, L., and Weidley, E., *Ann. N. Y. Acad. Sci.*, **66** 740(1957).
(4) Summa, A. F., and Jannke, P. J., THIS JOURNAL, **46**, 363(1957).
(5) Sakoda, J., Statistics Department, U. Conn., Personal Communication.
(6) Edwards, A. L., "Experimental Design in Psychological Research," Rinehart and Co., New York, N. Y., 1954, pp. 150–151.

The Antidotal Effectiveness of Sodium Cobaltinitrite in Antagonizing Cyanide Poisoning in Albino Mice*

By MARVIN M. GOLDENBERG† and DAVID E. MANN, Jr.

The prophylactic intraperitoneal administration of sodium nitrite (80 mg./Kg.) thirty minutes prior to the subcutaneous injection of one LD_{95} of sodium cyanide protected 80 per cent of the mice. Under similar circumstances, sodium cobaltinitrite (60 mg./Kg.) afforded optimal protection to 97 per cent. Both sodium nitrite and sodium cobaltinitrite, administered thirty seconds after the injection of 1 LD_{95} of sodium cyanide, required lower doses and were more effective than when given prophylactically. The most effective therapeutic dose of sodium nitrite was 50 mg./Kg. which produced 90 per cent survival, while sodium cobaltinitrite was most effective at a dose of 30 mg./Kg., which produced a 96 per cent survival. Sodium cobaltinitrite–sodium thiosulfate combinations, when antidoting 4 LD_{95}'s of sodium cyanide, achieved percentage results that were similar to those attained with sodium nitrite–sodium thiosulfate combinations in antidoting 3 LD_{95}'s of the poison.

DESPITE A DECLINE in the incidence of accidental and suicidal cyanide deaths during the past twenty-five years (1), the search for more effective cyanide antidotes continues. Hydrogen peroxide (2), potassium permanganate (3), glutathione and cystine (4), cobalt nitrate (5), methylene blue (6), *p*-aminopropiophenone (7), and vitamin B_{12} (8) are valid antagonists experimentally, but these agents failed to protect against as many median lethal doses as either sodium nitrite or sodium thiosulfate, while the combination of sodium nitrite and sodium thiosulfate, as demonstrated by Chen and Rose (9) in dogs, proved capable of antagonizing twenty median lethal doses of sodium cyanide, and accordingly was modified for clinical use.

The inception of cobalt salts as cyanide antagonists occurred in 1894 (10) when Antal protected a person against hydrocyanic acid poisoning with the oral and subcutaneous administration of co-

* Received August 21, 1959, from Temple University, School of Pharmacy, Philadelphia 40, Pa.
† Present address: Hahnemann Medical College, Philadelphia, Pa.
Abstracted from a thesis presented to the Graduate School of Temple University, School of Pharmacy, by Marvin M. Goldenberg in partial fulfillment of the requirements for the degree of Master of Science.
Presented to the Scientific Section, A. PH. A., Cincinnati, meeting, August 1959.

balt nitrate. The subsequent decline in the use of cobalt salts was attributed to the introduction of other antidotes and a report by Martin and O'Brien in 1901 (11) that these agents offered no advantage over ferrous salts which, incidentally, were less toxic. In 1935, a Russian investigator (12) prophylactically protected mice against lethal doses of sodium cyanide with cobalt nitrate and cobalt sulfate. Since nitrites and cobalt salts have been demonstrated to be methemoglobin formers and sustainers, respectively, it was presumed that sodium cobaltinitrite might be useful in cyanide poisoning.

The primary purpose of this investigation was to compare sodium cobaltinitrite with sodium nitrite in terms of antidotal effectiveness when each agent was administered alone, prophylactically and therapeutically, and in conjunction with sodium thiosulfate.

EXPERIMENTAL

Adult albino mice (Huntingdon Farms HTF strain), of mixed sex, weighing between 19.0 and 34.5 Gm. were employed in this study. The following concentrations of each agent were prepared in distilled water: sodium cyanide, 0.1 to 0.4%; sodium nitrite, 0.3 to 1.0%; sodium cobaltinitrite, 0.1 to 1.0%; and sodium thiosulfate, 25%. Solutions of sodium cyanide were administered subcutaneously, while the antidotes were injected intraperitoneally. Cessation of respiration was the criterion of death. Observations were made over a twenty-four-hour period.

Determination of the LD_{95} of Sodium Cyanide.—One hundred and forty-three mice were divided into five groups containing 12, 15, 6, 10, and 100 animals. Doses of sodium cyanide, ranging from 14 to 18 mg./Kg. were administered to each group in increasing increments of 1 mg./Kg. to determine a dose that would cause 100% mortality. Since only 100 animals were tested, it would be statistically incorrect to use the term minimal lethal $dose_{100}$ (MLD_{100}).

Establishment of the Minimal Prophylactic Dose of Sodium Cobaltinitrite Against 1 LD_{95} of Sodium Cyanide.—The evaluation of the effectiveness of sodium cobaltinitrite as a potential cyanide antagonist entailed the establishment of an antidotal dose which possessed a wide margin of safety. Furthermore, the optimal time interval between the administration of the antidote and the poison had to be ascertained, for methemoglobinemia produced by nitrite ion and maintained by cobalt ion (13) may attain a maximal concentration within several minutes and then drop sharply causing death from cytotoxic anoxia. Three hundred mice, half males and half females, were divided into three equal groups. Freshly prepared solutions of sodium cobaltinitrite, in concentrations of 0.1 and 1.0%, were administered to each group in successively increasing doses of 40, 50, and 60 mg./Kg., followed in thirty minutes by the administration of 1 LD_{95} of sodium cyanide.

Establishment of the Minimal Prophylactic Dose of Sodium Nitrite Against 1 LD_{95} of Sodium Cyanide.—The experimental plan in this determination was identical to that of the previous procedure except that freshly prepared solutions of 0.5% sodium nitrite were administered to three groups of 100 mice each in doses of 60, 70, and 80 mg./Kg.

Relative Therapeutic Effectiveness of Sodium Cobaltinitrite and Sodium Nitrite Against 1 LD_{95} of Sodium Cyanide.—The purpose of this procedure was to determine the optimal therapeutic dose for each antidote against 1 LD_{95} of sodium cyanide when the poison was administered initially, followed in thirty seconds by either agent. Varying concentrations of sodium cobaltinitrite and sodium nitrite were prepared as follows according to the dose: 0.3% when the dose was 30 mg./Kg.; 0.4% for 40 mg./Kg.; and 0.5% when the dose was either 50, 60, or 90 mg./Kg. Sodium cyanide was administered as a 0.1% solution. Groups of fifty mice, of equally divided sex, were employed.

Relative Therapeutic Effectiveness of Sodium Nitrite–Sodium Thiosulfate and Sodium Cobaltinitrite–Sodium Thiosulfate Against Increasing LD_{95}'s of Sodium Cyanide.—Chen and Rose (9) established that sodium nitrite could antidote four median lethal doses of sodium cyanide in dogs. When sodium thiosulfate was administered in conjunction with sodium nitrite, marked potentiation occurred which increased the number of LD_{50}'s of cyanide to twenty. This study was undertaken to observe the effect of antidotal combinations against increasing LD_{95}'s of sodium cyanide. Concentrations of sodium cyanide, from 0.1 to 0.4%, were prepared and administered when the LD_{95}'s were increased from one to four. Sodium cobaltinitrite and sodium nitrite, as 0.5% solutions, were administered in doses of 60 mg./Kg. A 25% solution of sodium thiosulfate, 0.2 ml., was administered to each animal as a fixed dose. Each group consisted of fifty mice of equally divided sex. The sequence of injections was as follows: sodium cyanide was injected subcutaneously, sodium cobaltinitrite or sodium nitrite was given intraperitoneally immediately following the cyanide, sodium thiosulfate was then administered at once intraperitoneally in another portion of the abdomen.

RESULTS

LD_{95} of Sodium Cyanide.—The administration of sodium cyanide to four groups of mice in doses of 14, 15, 16, and 17 mg./Kg. produced survivals of 58% at the lower dosage level and 30% at the higher level. No survivals occurred at 18 mg./Kg. which was accordingly designated the LD_{95} of sodium cyanide because of the limited number of animals used.

Minimal Prophylactic Dose of Sodium Cobaltinitrite Against 1 LD_{95} of Sodium Cyanide.—Thirty minutes was chosen as the optimal time interval between the injection of sodium cobaltinitrite and sodium cyanide, for this period appreciably increased per cent survival as well as prolonged survival time. The administration of sodium cobaltinitrite thirty minutes prior to the cyanide at a dose of 40 mg./Kg. resulted in a 47% survival with an average survival time of 50 minutes. At 50 mg./Kg., sodium cobaltinitrite produced an 85% survival with an average survival time of 56 minutes. The minimal prophylactic dose of the antidote was established at 60 mg./Kg., which produced a 97% survival with an average survival time of 58.3 minutes.

Minimal Prophylactic Dose of Sodium Nitrite Against 1 LD_{95} of Sodium Cyanide.—Sodium nitrite, administered prophylactically thirty minutes prior to sodium cyanide at a dose of 60 mg./Kg. produced a 13% survival with an average survival time of 49.9 minutes. At 70 mg./Kg. sodium nitrite increased the per cent survival to 49 and the average survival time to 53.3 minutes. The minimal prophylactic dose, established at 80 mg./Kg., caused an 85% survival with an average survival time of 45.3 minutes.

Therapeutic Effect of Sodium Cobaltinitrite and Sodium Nitrite Against 1 LD_{95} of Sodium Cyanide.—The administration of 1 LD_{95} of sodium cyanide followed in thirty seconds by either sodium cobaltinitrite or sodium nitrite at various dosage levels produced the following results: at 60 mg./Kg., sodium cobaltinitrite protected 100% of the mice, while sodium nitrite protected 70%. At 50 mg./Kg., sodium cobaltinitrite again afforded 100% protection, while sodium nitrite increased the survival percentage to 90. At 40 and 30 mg./Kg., sodium cobaltinitrite produced survivals of 98 and 96%, while sodium nitrite decreased the survival percentage to 74 and 42, respectively.

Sodium Nitrite-Sodium Thiosulfate and Sodium Cobaltinitrite Sodium Thiosulfate Against Increasing LD_{95}'s of Sodium Cyanide.—When each antidote was administered with a fixed dose of sodium thiosulfate immediately after 1 LD_{95} of sodium cyanide, sodium cobaltinitrite protected 100% of the mice, while sodium nitrite protected 92%. Against 2 LD_{95}'s, sodium cobaltinitrite again showed complete protection, while only 68% of the animals were protected by sodium nitrite. Against 3 LD_{95}'s, 80% of the mice survived with sodium cobaltinitrite protection, while only 14% were protected by sodium nitrite. Finally, against 4 LD_{95}'s, no animals survived with sodium nitrite, while 18% were protected by sodium cobaltinitrite.

DISCUSSION

Sodium cobaltinitrite, when administered alone prophylactically and therapeutically, and therapeutically with a fixed dose of sodium thiosulfate, proved to be a more effective antidote for cyanide poisoning in mice than sodium nitrite under similar conditions. Although sodium nitrite, when given thirty seconds after 1 LD_{95} (18 mg./Kg.) of sodium cyanide was more effective at a lower dosage level than that required for prophylactic usage, sodium cobaltinitrite produced more survivals at all therapeutic dosage levels. Thus, one may assume that the antidotal ineptness of sodium nitrite is due to the production of methemoglobinemia of short duration, while sodium cobaltinitrite apparently has a more sustained action in perpetuating this state. This assumption is based upon the work of Shen, *et al.* (13), who elucidated the probable mechanism of action of cobalt on the reversible hemoglobin-methemoglobin system. They concluded that cobalt inhibited the enzymatic reduction system concerned with the conversion of methemoglobin to hemoglobin and that the increased formation of methemoglobin was not due to the acceleration of the oxidative process beyond the capacity of the normal reduction mechanism. When they administered cobalt salts to experimental animals, increased levels of methemoglobin concurrent with a decrease in the oxygen-carrying capacity of the peripheral blood failed to appear. Therefore, one may conclude that a probable mechanism of detoxification for cobaltinitrite is the production and maintenance of methemoglobin due to the presence of the two different chemical ions.

In order for antidotes to work effectively, they should not only be capable of overcoming the toxic effects of the poison but also be relatively free of untoward effects when administered. Therefore, acute toxicity studies with sodium cobaltinitrite were observed upon a limited number of mice. It was found that the antidote produced an approximate LD_{50} of 140 mg./Kg. Ostreiko and Charausoff (5) reported that the LD_{50} of cobalt nitrate in rabbits was 60 mg./Kg., while Murdock and Klotz (14) found the LD_{50} of cobalt chloride in rats to be approximately 8 mg./Kg.

REFERENCES

(1) Mortality Statistics, Bureau of Census, U. S. Dept. of Commerce, 1926(1949).
(2) Krohl, P., *Arb. d. pharm. Inst. zu Dorpat.*, **7,** 153(1892).
(3) Kossa, W., *Ungar. med. Arch.*, **2,** 12(1893).
(4) Voegtlin, C., *J. Pharmacol. Exptl. Therap.*, **27,** 467(1926).
(5) Ostreiko, O., Charausoff, H. A., *Fiziol. Zhur. S.S.S.R.*, **21,** 643(1936).
(6) Brooks, M. M., *Am. J. Physiol.*, **102,** 145(1932).
(7) Rose, C. L., Welles, J. S., Fink, R. D., and Chen, K. K., *J. Pharmacol. Exptl. Therap.*, **89,** 109(1947).
(8) Mushett, W., *et al.*, *Proc. Soc. Exptl. Biol. Med.*, **112,** 234(1952).
(9) Chen, K. K., and Rose, C. L., *J. Am. Med. Assoc.*, **149,** 113(1952).
(10) Antal, J., *Ungar., med. Arch.*, **3,** 117(1894).
(11) Martin, C. and O'Brien, M., *Intercolon. M. J. of Austral.*, **6,** 245(1901).
(12) Rozhkov, V. M., *Fiziol. Zhur. S.S.S.R.*, **19,** 582(1935).
(13) Shen, C. J., *et al.*, *J. Clin. Invest.*, **33,** 1560(1954).
(14) Murdock, H. R., and Klotz, I. J., THIS JOURNAL, **48,** 143(1959).

A Comprehensive Pharmaceutical Stability Testing Laboratory III*

A Light Stability Cabinet for Evaluating the Photosensitivity of Pharmaceuticals

By LEON LACHMAN, CHARLES J. SWARTZ, and JACK COOPER

The design and utilization of a light stability cabinet for testing pharmaceuticals has been described. The lighting system developed for this cabinet is intended to simulate ordinary room illumination found in the pharmacy, home, doctor's office, or in other storage areas for pharmaceuticals, but under accelerated light intensity. The use of this equipment eliminates the influence of certain wavelengths in the ultraviolet spectrum of artificial sunlight testing equipment presently in use. Several representative pieces of artificial sunlight testing equipment used in the textile and dye industries are discussed. The photosensitivity of two tablet preparations were studied utilizing the specially designed light stability cabinet. Samples were stored in an open dish and in amber bottles. In one case the tablets contained a sulfonamide which darkened, while the other tablets contained a benzothiadiazine derivative plus D&C Orange No. 3 which showed fading of the color with storage. It was demonstrated that the amber glass appears to inhibit the deleterious effects of light on the tablets.

THE USE of more complex active constituents and certified colorants in pharmaceutical dosage forms in recent years has focused increased attention on the influence of light on the stability of these products. As a result, manufacturers of pharmaceuticals have attempted to utilize various exaggerated light testing techniques in an effort to predict the degree of photosensitivity for new formulations. However, although it is known that tests of this nature have become more prevalent, few reports exist in the literature concerning their use. This may be attributed to the fact that the methods employed were not completely satisfactory, were highly specific, and did not simulate the spectral energy distribution of ordinary room illumination.

Because of the apparent lack of satisfactory equipment for light study evaluations of pharmaceuticals, an investigation was undertaken to develop a suitable apparatus. As a result, a cabinet was designed to permit the study of dosage forms under illumination exhibiting a radiant energy distribution similar to that of ordinary room lighting, but under exaggerated light intensity. This paper reports on the photosensitivity of two tablet formulations, one containing a sulfonamide and the other a benzothiadiazine derivative plus D&C Orange No. 3. The samples were stored in the light stability cabinet so that the intensity of light falling on the products was approximately 550 foot candles. The protective influence of amber glass containers on the tablets stored in the light cabinet was ascertained.

PREVIOUS STUDIES

Reports in the pharmaceutical literature concerning the influence of light on the stability of dosage forms are rare. Experiments performed by Arny, *et al.* (1, 2), disclosed the effects of sunlight and diffused light on various pharmaceutical ingredients when stored in several types of glass containers. Of all the commercially available glasses studied, they found that amber glass afforded the greatest protection. The authors studied the deterioration of 50 medicaments and summarized the causes as follows: (*a*) light, (*b*) simple volatilization, and (*c*) chemical changes produced by factors other than light. Garrett and Carper (3) reported on color stability of a liquid multisulfa preparation as affected by thermal degradation. The influence of several pharmaceutical materials on the fading of FD&C Blue No. 2 in solution was investigated by Kuramoto, *et al.* (4). As in the previous reference (3) however, no attempt was made to isolate the contribution of the light intensity factor to the overall reaction.

Although there is a conspicuous lack of information concerning the light stability of pharmaceuticals, there appears to be an abundance of information available on the influence of light on textiles, dyes, and pigments (5–14). The necessity of having a standard light fastness test is well recognized in these fields.

The most readily available source of radiant energy is sunlight, but it is hardly necessary to point out the unsuitability of sunlight or daylight as a source of light in testing color stability. The radiation received from the sun is subject to wide variations both in total intensity and in distribution of energy. Because of this, exposure tests to sunlight result in marked differences in relative effect for samples exposed to this light source. It has been re-

* Received June 11, 1959, from the Research Department, Ciba Pharmaceutical Products Inc., Summit, N. J.
Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.

ported by Appel and Smith (10) that by limiting the exposure of samples to between 9 a. m. and 3 p. m. on clear days, the low intensity radiation and high atmospheric humidity occurring at intervals during continuous exposure is avoided. This, they indicate, yields data that have been shown to be generally reproducible at different times of the year and in different parts of the United States. Despite the above effort at standardization of sunlight exposure, the findings of other investigators appear to be at variance with the findings of Appel and Smith since no compensation was made for the influence of clouds, mist, or time of year.

Attempts to measure the energy of the sun's rays (15) and to make exposures for a definite number of energy units were inadequate in that the following factors were not taken into consideration: (*a*) variation of sun intensity from season to season and area to area, (*b*) daily cycles of change in temperature, (*c*) daily cycles of change in humidity, (*d*) variations in spectral character of radiation during time of exposure, (*e*) the influences of gases such as ozone, oxides of nitrogen, and sulfur, and (*f*) the presence of other atmospheric impurities. In an endeavor to concentrate the sun's rays and thereby decrease the time required for fading exposure, lenses were utilized, though unsuccessfully because of the excessive heat developed (16). In order to control, diminish, or eliminate the effect of these variables, various artificial light sources have been developed with a spectral energy distribution intended to simulate that of direct sunlight. Sources such as carbon arcs (17, 18), mercury vapor lamps (19), fadeometers (6, 8, 11), daylight (10), and tungsten filament lamps (9, 12) have been used to determine the light stability of colored textiles, dyes, and chemicals susceptible to photochemical reaction.

The most commonly used reproducible high intensity point light source is the carbon arc lamp. This lamp is made in three types: flame arcs, low-intensity arcs, and high-intensity arcs. Flame arcs may be open to the air or enclosed in a glass globe. They are arranged so that a large proportion of their radiation is derived from the flame and only a comparatively small amount emitted from the tips of the electrodes. In all forms of carbon arc, the carbon electrodes burn away by oxidation and volatilization. In the enclosed arc, only a limited supply of oxygen reaches the carbons, with an ensuing burning rate which is very low. As a result, the enclosed carbon arc is the form most commonly employed in fading tests. This source resembles sunlight only to the extent that the proportion of radiant power emitted in the region 300–400 mμ is approximately the same as summer sunlight. The high intensity carbon arc is the nearest to noon sunlight in its spectral characteristics but this type of arc cannot be left to burn unattended for periods longer than a few hours. Since attempts are made to extrapolate one hundred to two hundred hours of this exposure data to several years, operation irregularities can cause large errors in extrapolated results. Further, as the high intensity arc lamp burns, the flame rotates slowly around the electrodes and, at any one time, the intensity of illumination on one side of the lamp may be three times as great as that on the opposite side.

Another artificial source frequently employed would be mercury vapor discharge lamps, and a number of types of electric discharge lamps have been used for photochemical work. In each of these units the discharge passes through mercury vapor and the spectrum of mercury, which differs according to the operating conditions of the lamp, is emitted. In addition to supplying high intensities of irradiation, the spectrum is relatively rich in radiation throughout the blue and ultraviolet region. The advantages of this equipment are compactness, cleanliness, uniform intensity, and lower heat output for equal illumination. But the disproportionate amounts of ultraviolet light present in this source prevents the accumulation of useful information relevant to daylight fading.

A third principle light source would be the familar tungsten filament lamp. It consists of a coil of tungsten wire mounted in a glass bulb containing an inert gas. The wire is heated to incandescence by the passage of current and the spectrum is the usual continuous type characteristic of an incandescent body. The distribution of energy of a typical high-wattage filament lamp usually commences slightly below 400 mμ and increases at a rapid rate from there. The candle power of the lamp falls slowly throughout its life, due to the volatilization of the tungsten and increased resistance of the filament.

Cabinets for housing the light source, whatever its nature, are available in a multiplicity of designs. Numerous patents (20–24) have been issued for various installations dependent upon the desired end use. The basic design consists of a housing to contain the sample at a fixed distance from a light source. A typical apparatus generally comprises a hollow cylindrical casing enclosing a source of light and having about its periphery a series of sample holders. The Fade-Ometer, a widely employed standardized unit, is based on this principle. In some instances, provisions are made for humidity and/or temperature control, while in other cases only the character of the radiation is regulated. The degree of control varies with the intended end use of the data obtained, but in almost all situations an attempt is made to extrapolate the information derived to some extended time interval.

It is readily evident from the previous sections of this report, that the artificial light sources described were intended to simulate sun illumination under controlled environmental conditions. Hitherto, consideration has not been given to the possibility of utilizing a high intensity light source which would simulate ordinary room illumination. This type of light source should more closely approximate the spectral energy distribution falling on products kept in usual storage areas. Very seldom are products exposed to direct sunlight and when they are, it is for a relatively short period of time.

EXPERIMENTAL

Description of Cabinet for Accelerated Light Testing.—The magnitude of a photochemical reaction is dependent upon the amount of light energy absorbed by the material under study. The energy E absorbed by each reacting mole of material can be expressed by the following equation:

$$E = Nhc/\lambda$$

where N is the Avogadro number, c the velocity of light, h Plank's constant, and λ the wavelength of the absorbed radiation. Inserting the value N as 6.023×10^{23}, h as 6.624×10^{-27} and c as 2.9977×10^{10}, the energy is obtained in ergs; dividing by 4.184×10^{10} gives the value in Kcal.

$$E = 2.859 \times 10^{5}/\lambda \text{ Kcal. per mole}$$

It can be seen from the equation that the energy absorbed per mole becomes greater as the wavelength of the light is shortened. Therefore, the radiation in the ultraviolet and violet portions of the spectrum should be more chemically active than those of the longer wavelength (visible light). In general, photochemical activity drops off with increasing wavelength.

Since most pharmaceutical preparations are usually stored in retail pharmacies, storage areas of a warehouse, homes, hospitals, and doctor's offices, the amount of light in the ultraviolet spectrum of sunlight that penetrates through the glass store front or window panes is substantially decreased because of absorption by the glass. Husa (25) indicated that ordinary glass absorbed the shorter ultraviolet light as well as the longer infrared found in sunlight. This would suggest that the decomposition caused by sunlight passing through glass would be due to visible light and/or the ultraviolet and infrared which are nearest in wavelength to visible light.

In a report by Esselen and Barnby (26) light intensities outside and inside retail stores were measured and compared. These readings were taken in a number of midwestern stores with unobstructed southern exposure at noon on clear days in the middle of June. The values obtained from such exposure should represent the most severe sunlight exposure conditions obtainable. Foot candle readings of intensity were made both inside and outside the store front with the meter pointing directly south. The data obtained from this study are reproduced in Table I. It is readily evident from the data that the outdoor readings were over 400 times the average reading taken on typical store shelves exposed to light of normal intensity.

TABLE I.—LIGHT INTENSITY OF TYPICAL RETAIL STORE LOCATIONS[a]

Meter Location	Average Light Intensity, Food candles	Outside Reading/ Inside Reading
Outside store windows	6,500	
Shelves, front of store	15–30	216 to 433
Shelves, middle of store	5–15	1,300 to 433
Shelves, shaded parts of store	1–5	6,500 to 1,300

[a] See reference 26.

In view of the preceding discussion, it would seem that in order to measure the effect of light on the sensitivity of pharmaceuticals accurately, a lighting system should be developed which simulates ordinary room illumination but under accelerated light intensity. Through the use of such equipment the influence of certain wavelengths in the ultraviolet spectrum of artificial sunlight sources which are generally not present in storage areas for pharmaceuticals would be eliminated.

It is desirable to utilize high intensity light for studies of a photochemical nature, since the chemical change produced by light is generally small and develops at a slow rate. For example, it has been calculated that radiation of one candle power falling on 1 sq. cm. corresponds to 2×10^{14} quanta per second, so that this number of molecules should react per second. The number of moles reacting per second per candle power is therefore 3.3×10^{-10} per sq. cm. of substance exposed to radiation (27).

In consideration of the above, a light stability cabinet was fabricated to reproduce ordinary room illumination, but under substantially intensified light. A brief preliminary discussion of this cabinet was presented in an earlier paper (28). Figure 1 gives a comprehensive description of this cabinet with the lighting system employed and its specifications.

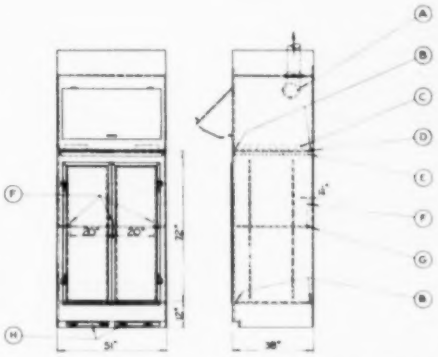


Fig. 1.—Comprehensive description of the lighting cabinet used for exaggerated light stability testing. *A*, Exhaust blower; *B*, slit front and rear (air supply); *C*, ballast, (9) No. 89G604-G.E.-150 watts, each ballast is wired with (2) No. 48T12 fluorescent tubes; *D*, Alzac aluminum sheet reflector above lamp tubes; *E*, rapid start lamps, (18), No. 48T12 CWRS, $1^1/_2$ inches in diameter $\times$ 48 inches long slant line cool white, G.E., rated for 3,250 lumens each 60 watt tubes. Ground tubes minimum of $^1/_2$ to 1 inch from mounting surface. (9) G.E. No. 505 $\times$ 91 socket, (9) G.E. No. 505 $\times$ 92 socket; *F*, (3) thermocouples on these center lines; *G*, Alzac aluminum shelf (adjustable); *H*, grill for air supply.

The fluorescent tubes used are rated for 3250 lumens each and are of the slant line cool white, rapid starter type. They are fabricated to give off the least amount of heat per foot candles of light intensity produced. Any heat that emanates from the fluorescent tubes is removed by means of a blower located above the ballasts containing the tubes. A curve representing the spectral distribution of energy in typical sunlight as it reaches the earth's surface and in the emission of a "standard cool white" fluorescent lamp is presented in Fig. 2. The curves have been adjusted so that each represents the same amount of light. It can be seen that the ratio of ultraviolet radiation to visible light is greatest for sunlight and substantially less for the "standard cool white" lamp. Nevertheless, it should be emphasized that light intensity within buildings is manyfold less than in direct sunlight.

Alzac aluminum has been used for the sample tray

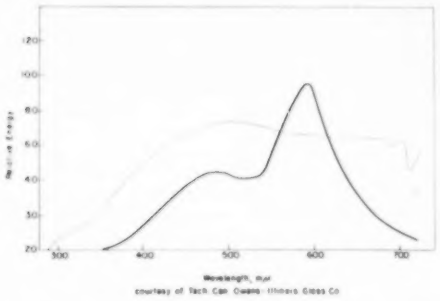


Fig. 2.—Spectral energy distribution of sunlight and standard cool white fluorescent lamp. . . . . Sun, ——— standard cool white fluorescent.

as well as behind the fluorescent tubes for reflectance purposes.

The ballasts provide for proper starting voltage and also control the operating voltage and current in the tubes.

The shelf in the cabinet is adjustable so that it can be moved to any desired height. This adjustment makes it possible to obtain variable light intensities, since the light intensity is apparently inversely proportional to the square of the distance.

Through the use of this cabinet it is possible to obtain a reproducible light intensity over a definite spectral range at any time during the year under controlled conditions of temperature and humidity. This should permit the determination of the stability of pharmaceuticals with regard to light in a relatively short period of time.

Description of Light Stability Cabinet for Ordinary Illumination.—This cabinet has been fabricated to simulate room illumination intensity and spectral energy distribution. Samples are stored in this cabinet at the same time they are exposed to accelerated light intensity. The dimensions of this cabinet are 37 inches high by 18 inches deep by 30 inches long. The light sources in this cabinet are two 8-watt and two 15-watt fluorescent tubes which can be used independently or in combinations to attain the desired light intensity. The interior of the cabinet is painted a flat white for reflectance purposes. The cabinet permits the exposure of samples to room illumination under specified conditions and acts as a control for exaggerated intensity studies.

The light meter employed for intensity measurements is a Gossen Tri Lux foot candle meter model C which has a range of measurement from 0–12,000 foot candles. It is an incident meter measuring the light falling on an object. The accuracy of this meter is ±5% of the end scale value.

Procedure for Testing Products.—Samples of two different tablet formulations, one containing a sulfonamide and the other a benzothiadiazine plus D&C Orange No. 3 as colorant were exposed to 550 foot candles of light intensity. These tablets were held in an uncovered container and in an amber bottle. Reflectance measurements were performed on the tablets at designated time intervals utilizing a model DU Beckman spectrophotometer with a reflectance attachment. Special dies were prepared to hold the tablets in the reflectance attachment. Reflectance measurements for the sulfonamide tablet were performed at 450 mμ and for the tablet containing the benzothiadiazine at 500 mμ.

Results and Discussion.—The light intensity falling on the materials being tested was maintained at a constant level by regular measurement with the Gossen foot candle meter followed by necessary adjustments in the shelf distance from the source of light. In an earlier report (28) it was shown that it is possible to calculate the foot candles of light intensity falling on a test material with the aid of a suitable equation.

A summary of the results obtained for the sulfonamide tablets is presented in Table II. It is evident from this data that the tablets darken with storage since the reflectance values decrease substantially. Visual observation of these tablets indicate that they have taken on a yellow-tan color. The darkening of the tablets can probably be ascribed to oxidation of the sulfonamide which is potentiated by the light. However, for samples stored in amber bottles no significant change in reflectance values from the original 52.6% was noted. The amber glass appears to protect the sulfonamide tablets from the harmful effects of the light in the cabinet. The data in this table were plotted on semilog paper and the curves are shown in Fig. 3. By plotting the log of the per cent reflectance vs. time, straight line curves are obtained. For the samples exposed directly to the intense light a curve results which has two slopes, the first being steeper than the second.

The effect of light on the tablets containing the benzothiadiazine derivative colored with D&C

TABLE II.—INFLUENCE OF ACCELERATED LIGHT STORAGE ON THE PHOTOSENSITIVITY OF A SULFONAMIDE TABLET FORMULATION

Time, Wks.	Reflectance at 450 mμ, %: Open Dish	Amber Bottle
0	52.6	52.6
2	41.3	52.3
4	35.6	53.6
6	31.7	53.8
8	28.9	52.9
10	25.4	. . .
12	23.8	51.5

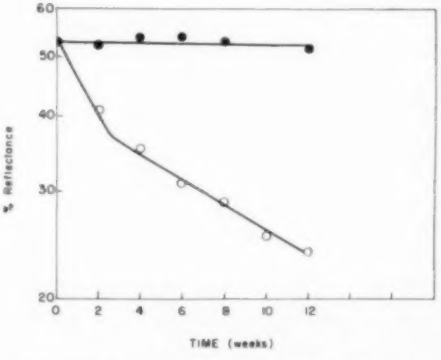


Fig. 3.—A plot showing the influence of light on the color stability of a sulfonamide tablet. ● Tablets stored in amber bottle, ○ tablets stored in open dish.

TABLE III. INFLUENCE OF ACCELERATED LIGHT STORAGE ON THE PHOTOSENSITIVITY OF A TABLET FORMULATION CONTAINING A BENZOTHIADIAZINE DERIVATIVE AND D&C ORANGE NO. 3

Time, Wks.	Absorbance at 500 mμ	
	Open Dish	Amber Bottle
0	0.302	0.302
2	0.234	0.302
4	0.212	0.315
6	0.203	0.304
8	0.188	0.312
10	0.198	0.312
12	0.190	0.309

Orange No. 3 is shown in Table III. It can be readily seen from the data in this table that the color of the tablets exposed directly to the intense light fades significantly as indicated by an increase in reflectance values. Again, the samples stored in amber bottles show essentially no change with storage. By plotting the logarithm of the absorbance *vs.* time, straight line curves are obtained as shown in Fig. 4. The plot for the sample exposed directly to the intense source of light shows three different slopes. Since the plots are straight lines, it would seem that the fading follows an apparent first-order reaction. Between seven and eight weeks, the curve levels off and shows no further fading. Because of these interesting data, a more comprehensive study was initiated concerning the influence of ordinary room illumination and exaggerated lighting on the rate of fading of several commonly used certified colorants. The results obtained from this study will be presented in a separate report.

The protective characteristics of amber glass becomes readily evident from the transmittance curves for flint and amber glass shown in Fig. 5. While flint glass transmits significantly from 300 mμ, the amber glass does not begin to transmit to any appreciable extent until 470 mμ. This would indicate that the energy emitted by cool white fluorescent lamps in the light cabinet beyond 470 mμ does not exert any appreciable effect on the samples tested. Since it is believed that reactions of particular chemicals are effected by a narrow band of wavelengths near the absorption maximum of the chemical (11), it may be anticipated that the influence of the light source would vary according to the effective range of wavelengths present.

A more complete study on the influence of various colored glass and thickness on the light stability of a number of pharmaceutical components is under way and will be reported on in the near future.

According to the data presented in this report as well as that obtained from additional studies performed in our laboratories, it would appear that although a relationship exists between the extent of a reaction and light intensity of ordinary and exaggerated illumination, this relationship is not independent of the material undergoing testing. In other words, the exaggeration produced by the high light intensity for one chemical could be tenfold while for another chemical only threefold. However, the extent of the reaction for each chemical is generally assumed to be dependent on the product of time and light intensity (11). These differences in degree of exaggeration that are produced for different chemicals can be ascribed to several factors: (*a*) the wavelengths of the spectrum which are absorbed, (*b*) the amount of electromagnetic radiation of a suitable wavelength required to cause the molecules of a chemical to reach its excited state, and (*c*) the amount of radiation absorbed. Therefore, it seems most appropriate to determine the extent of exaggeration of a reaction produced by the high light intensity employed in the light stability cabinet for each product studied and not attempt to apply data from previous tests on similar products until sufficient studies have been performed to indicate the feasibility of this approach.

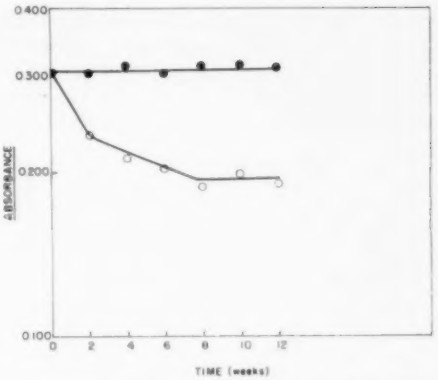


Fig. 4.—The effect of light on the fading of a tablet colored with D&C Orange No. 3. ● Tablets stored in amber bottle, ○ tablets stored in open dish.

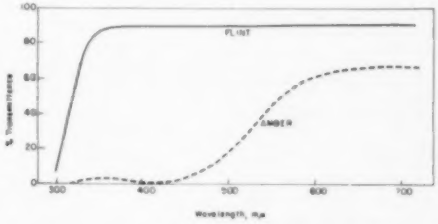


Fig. 5.—Transmission curves for typical flint and amber glass.

SUMMARY AND CONCLUSIONS

The design and application of a light stability cabinet for testing the photosensitivity of pharmaceutical products has been described. The lighting system employed for this cabinet is believed to simulate ordinary room illumination but under accelerated light intensity.

Data are presented for the effect of light on the stability of a sulfonamide and a benzothiadiazine tablet formulation. The colorant used for the benzothiadiazine tablet was D&C Orange No. 3.

The influence of amber glass on the light stability of the two tablet formulations was deter-

mined. It was shown that although the lighting in the cabinet exerted significant deleterious effects on the color of the tablets stored in an open dish, these changes were virtually prevented when the tablets were stored in amber containers.

REFERENCES

(1) Arny, H. V., Taub, A., and Blythe, R. H., THIS JOURNAL, **23,** 672(1934).
(2) Arny, H. V., Taub, A., and Steinberg, A., *ibid.*, **20,** 1014(1931).
(3) Garrett, E. R., and Carper, R. F., *ibid.*, **44,** 515 (1955).
(4) Kuramoto, R., Lachman, L., and Cooper, J., *ibid.*, **47,** 175(1958).
(5) Lips, H. A., *Paper Trade J.*, **120,** 108(1954).
(6) Nordhammer, G., and Gralen, N., *J. Soc. Dyers Colourists*, **65,** 741(1949).
(7) Cady, W. H., *Am. Dyestuff Reptr.*, **20,** 130(1931).
(8) Cady, W. H., and Appel, W. D., *ibid.*, **18,** 407(1929).
(9) Luchiesh, M., and Taylor, A. H., *ibid.*, **14,** 613(1925).
(10) Appel, W. D., and Smith, W. C., *ibid.*, **17,** 410(1928).
(11) Morton, T. H., *J. Soc. Dyers Colourists*, **65,** 597 (1949).
(12) Cooper, B. S., and Hawkins, F. S., *ibid.*, **65,** 586(1949).
(13) Vickerstaff, T., and Tough, D., *ibid.*, **65,** 606(1949).
(14) Hadfield, I. H., *J. Textile Inst.*, **18,** 527(1927).
(15) Appel, W. D., *Am. Dyestuff Reptr.*, **16,** 715(1927).
(16) Gordon, C., *Textile Colorist*, **43,** 29(1921).
(17) Ollinger, C. G., *Am. Dyestuff Reptr.*, **31,** 28(1942).
(18) Lead, W. L., *J. Soc. Dyers Colourists*, **65,** 723(1949).
(19) Blaisdell, B. E., *ibid.*, **65,** 618(1949).
(20) Buttolph, L. J., U. S. pat. 1,818,687, August 11, 1931.
(21) British pat. 435,097, September 13, 1935.
(22) British pat. 390,731, April 13, 1933.
(23) British pat., 325,112, February 13, 1930.
(24) Stott, L., U. S. pat. 1,664,332, March 27, 1928.
(25) Husa, W. J., *Glass Container*, **7,** 9(1928).
(26) Esselen, W. B., and Barnby, H. A., *Modern Packaging*, **1939,** September.
(27) Glasstone, S., "Textbook of Physical Chemistry," D. Van Nostrand Co., Inc., New York, N. Y., 1954, p. 1159.
(28) Lachman, L., and Cooper, J., THIS JOURNAL, **48,** 226(1959).

A Preliminary Pharmacologic Investigation of the Roots of *Bixa orellana**

By NORRIS W. DUNHAM and KENTON R. ALLARD

Pharmacologic studies were made of a partially purified water extract of the roots of *Bixa orellana*. Tests showed that the extract produces a marked depressant effect on voluntary activity without impairing involuntary activity. It also possesses antisecretory, antispasmodic, and hypotensive activity.

BIXA ORELLANA is a plant indigenous to Central and South American countries. This plant is also called Achiote and has been used as a source of annatto, a yellow-red pigment, which is obtained from the seeds. The bush will grow to a height of 20 feet and is cultivated to yield the pigment and to serve as a wind-breaker at the edges of coffee and cocoa plantations. So far as is known, there has been no previous attempt to extract chemical or pharmaceutical products from the roots.

EXPERIMENTAL

Extraction Procedure.—The whole root was gathered, ground, and extracted in a large Soxhlet apparatus with various solvents. After defatting with petroleum ether and ethyl ether, the sample was extracted with 95% ethyl alcohol for twelve hours and the solvent evaporated spontaneously. A 4% yield of a brown powder with a melting point of 60–70° was obtained. The extraction process was repeated for twelve hours employing distilled water. The water was then evaporated with the aid of a flash evaporator and finally in a vacuum desiccator under partial vacuum. A 7% yield of a brown powder with a melting point of 180–190° was obtained.

Effects on Spontaneous Motor Activity.—The Actophotometer (Metro Industries) was employed in this study. Six groups of 6 male, albino mice of the Rockland-Swiss strain were injected intraperitoneally at each of 4-dose levels. Thirty minutes after injection of an aqueous solution of the water extract, the group was placed in the photocell activity cage and their movements were recorded for the first five minutes only. The testing was repeated on different mice injected intraperitoneally with an aqueous solution of chlorpromazine HCl. Control experiments were made at random times during the tests with intraperitoneal injections of 0.2 ml. of distilled water.

The percentage inhibitions were calculated and the ED_{50} (effective dose$_{50}$) were interpolated from a dose-response curve constructed on semilogarithmic paper relating log dose and percentage inhibition. The results are shown in Table I.

TABLE I.—CENTRAL NERVOUS SYSTEM ACTIVITY (MICE, I. P.)

Drug	Dose, mg./Kg.	Photocell	Roller
Bixa-water extract	21	ED_{50}	On
Chlorpromazine HCl	2	ED_{50}	Off

* Received August 21, 1959, from the Division of Pharmacy, Ferris Institute, Big Rapids, Mich.
Supported, in part, by a grant from the Upjohn Co.
Roots supplied by Harold Hartgerink, Holland, Mich.
Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.

Effects on Rolling Roller Activity.—The rolling roller apparatus originally reported by Dunham and Miya (1) as a method for detecting neurotoxicity has more recently been reported by Kinnard and Carr (2) as a method of distinguishing between various types of tranquilizing effects. At a roller speed of 5 r.p.m., a normal animal can maintain its equilibrium for an indefinite period. Certain central nervous system depressants will result in the animal being unable to remain on the roller for the two-minute test period.

This testing was done immediately after removing the mice from the Actophotometer. The results are also presented in Table I. It is evident from the table that the dose of chlorpromazine required to inhibit the spontaneous motor activity by 50% also incapacitates the animal to the extent that it cannot remain on the roller. In contrast to this result, the ED_{50} dose of the water extract of Bixa does not exhibit the undesirable central nervous system depression. According to this method of testing, toxicity of the water extract is not observed until the lethal dose range is reached.

Effects on Unstimulated Gastric Secretions.—A method similar to that reported by Risley, *et al.* (3), was followed employing six albino rats per dose. All drugs were injected intraduodenally in a dose of 400 mg./Kg. at the time of pyloric ligations. All drugs were administered suspended in 2% tragacanth and the controls received comparable amounts of the suspending agent only. Five hours after the operation, the animals were sacrificed and the gastric juice was collected. The effects on volume, free acidity, and total acidity are shown in Table II.

It is evident that the whole root and the fractions will inhibit the volume of gastric secretion. However, only the whole root and the alcohol fraction have an effect on acidity.

TABLE II.—ANTISECRETORY EFFECTS (RATS, 400 MG./KG., I. D.)

Drug	Mean Vol. Secreted, ml.	Mean Free Acidity, Meq./L.	Mean Total Acidity, Meq./L.
Controls	6.4	41	58
Whole root	3.1	15	32
Water extract	3.4	35	59
Alcohol extract	1.6	10	35

Effects on Intestinal Motility, In Vitro.—Segments of guinea-pig ileum were suspended in a 50-ml. aerated bath of Tyrode's solution at 38°. The spontaneous contractions were recorded kymographically. The addition of 50 mg. of the water extract resulted in a decrease in tonus and a cessation of contractions. At the end of approximately one minute, the rate and amplitude of contractions returned to normal but the tonus was still decreased. A similar pattern was noted when 100-mg. doses were added to the bath.

Effects on Blood Pressure.—Rats were anesthetized by the intraperitoneal administration of pentobarbital sodium (50 mg./Kg.). The carotid artery was cannulated and connected to a mercury manometer with polyethylene tubing filled with a solution of heparin in normal saline. The solutions of the water extract were injected into the femoral vein. Doses of 10 mg./Kg. and 25 mg./Kg. resulted in transient drops in blood pressure. However, doses of 50 mg./Kg. and 100 mg./Kg. resulted in the pressure falling to 40% of normal and remaining there for an average of thirty minutes. The pressure then gradually increased and was normal again after sixty to ninety minutes.

Miscellaneous CNS Testing.—Intraperitoneal doses of 20 mg./Kg. of the water extract did not potentiate pentobarbital-induced sleep time in mice.

Swinyard's techniques (4) using maximal electroshock and pentylenetetrazol were employed in testing the water extract for anticonvulsant properties. Intraperitoneal doses of 100 mg./Kg. in mice did not alter the convulsions produced which indicates absence of anticonvulsant activity.

Acute Toxicity.—Albino male mice, 18–22 Gm. of the Rockland-Swiss strain were employed for this determination. All animals were fasted but received water *ad libitum* for twelve hours prior to the test. The water extract was injected intraperitoneally and the animals were kept under observation for five days since some delayed deaths were noted two and three days after injection. At the end of this period, deaths were counted and the LD_{50} was determined by Behrens' method (5). The LD_{50} was calculated to be 700 mg./Kg.

SUMMARY AND CONCLUSIONS

These studies indicate the presence of pharmacologically active principles in the roots of *Bixa orellana*. The water extract has a pronounced depressant effect on voluntary activity (spontaneous motor activity) in the mouse without impairing the involuntary activity (rolling roller performance).

The intraperitoneal ED_{50} on depressing spontaneous motor activity is 21 mg./Kg. as compared to the intraperitoneal LD_{50} of 700 mg./Kg.

This extract inhibits the volume of unstimulated gastric secretions without influencing the acidity. It also possesses antispasmodic and hypotensive properties.

Anticonvulsant and potentiation of barbiturate-induced sleep activities are absent.

REFERENCES

(1) Dunham, N. W., and Miya, T. S., THIS JOURNAL, **46**, 208(1957).
(2) Kinnard, W. J., and Carr, C. J., *J. Pharmacol. Exptl. Therap.*, **121**, 354(1957).
(3) Risley, E. H., *et al.*, *Am. J. Physiol.*, **150**, 754(1947).
(4) Swinyard, E. A., THIS JOURNAL, **38**, 201(1949).
(5) Behrens, B., *Arch. exptl. Pathol. Pharmakol.*, **140**, 237 (1929).

An Investigation of Some Pharmaceutical Applications of Certain Fatty Acid Esters of Sucrose*

By HOWARD HOPKINS† and LA VERNE D. SMALL

The usefulness of sucrose monomyristate and sucrose dipalmitate in formulating oral preparations has been studied. The monoester, being quite hydrophilic, is useful as an emulsifier. It is not, however, sufficiently hydrophilic to solubilize volatile oils. The diester, though water-insoluble, is dispersible in hot syrups yielding gel-like products suitable for use as vehicles. Although both the monoester and the diester are claimed to be nontoxic orally, the monoester exhibits toxic effects parenterally. The intraperitoneal LD_{50} in the rat was determined. An improvement in the method of synthesis of the monoester is described. Formulations for preparation of the gel-like vehicles are suggested.

RECENT DEVELOPMENTS in sucrochemistry (1–21) have made available a new group of compounds known as the sucrose esters of the higher fatty acids. More specifically, these substances are mono- and diacyl derivatives of sucrose where the acyl group contains from 12–18 carbon atoms in the aliphatic chain (22–24). These new substances are nonionic, surface-active materials. The claim that these compounds were tasteless and nontoxic (1, 3, 6–11, 13, 14, 24–28), in contrast with hitherto available surfactants (26, 29–31), was suggestive that these new compounds might be especially useful in the food and pharmaceutical industries as emulsifiers, solubilizers, and dispersing agents. In pharmaceutical practice, these esters might (*a*) aid in overcoming some of the problems in the solubilization of flavoring materials and other chemicals occurring in pharmaceutical products, (*b*) serve as emulsifying agents in preparing emulsions for oral and parenteral use, and (*c*) provide the basis for the development of a vehicle or carrier for therapeutic agents.

This study was initiated to investigate the range of usefulness of certain of these compounds in the formulation of pharmaceutical products intended for oral administration. Two of these esters, sucrose monomyristate and sucrose dipalmitate, have been studied. The latter material was procured from a commercial source[1] but the former was synthesized in this laboratory using a modification of the procedure proposed by Osipow, *et al.* (15). Sucrose monomyristate, a water-soluble, nonionic surfactant, was studied for its ability to act as an emulsifying and solubilizing agent. The growth behavior of a number of microbial agents in solutions of sucrose monomyristate was examined for the purpose of determining whether this sugar ester would be readily attacked by some commonly encountered types of contaminants. The intraperitoneal LD_{50} for the rat was determined for sucrose monomyristate. Sucrose dipalmitate, a water-insoluble compound, was found to be useful in formulating flavorable, gel-like compositions suitable as vehicles for a variety of medicaments.

EXPERIMENTAL

Synthesis of Sucrose Monoester.—The Osipow procedure (15) for synthesizing the sucrose monoesters involves reacting, under reduced pressure, the methyl ester of the appropriate fatty acid with sucrose in a solvent of dimethylformamide in the presence of an alkaline catalyst. At completion of the reaction, the solvent is removed by distillation under reduced pressure, the residue is dissolved in water, and this solution is then extracted with 1-butanol. Distillation of the 1-butanol from this extract leaves a residue which, on repeated reprecipitation from acetone, supposedly yields the desired sucrose monoester. In this laboratory, this procedure always gave final products which were bitter. By making the aqueous solution alkaline (pH 11) prior to extraction with 1-butanol and by extracting the 1-butanol solution with acidulated water (pH 1), a tasteless sucrose monomyristate was obtained in a 17% yield. It was found that the soap, present in the residue obtained after distillation of the 1-butanol, could be removed from the reaction mixture residue if a sufficiently large (15 to 1) amount of acetone were used in the reprecipitation of sucrose monomyristate. At a 5–10% concentration of

* Received August 21, 1959, from the College of Pharmacy, University of Nebraska, Lincoln.
Abstracted from a thesis submitted to the Graduate College of the University of Nebraska in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
† Fellow of the American Foundation for Pharmaceutical Education. College of Pharmacy, University of Nebraska, Lincoln. Present address: College of Pharmacy, University of Kentucky, Lexington.
The authors wish to thank Dr. C. E. Georgi for his counsel and aid in the microbiological tests and to acknowledge with gratitude the helpful suggestions and assistance of Dr. R. D. Gibson in the toxicity determination.
Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.
[1] Millmaster Chemical Corp., 295 Madison Ave., New York 17, N. Y.

sucrose monomyristate in hot acetone, the soap is not solubilized and may be filtered off.

Efforts to synthesize sucrose monopalmitate from sucrose and sucrose dipalmitate, in the manner suggested for an alternate procedure (15), were wholly unsuccessful.

Sucrose Monomyristate as a Solubilizer.—One-milliliter quantities of a number of volatile oils, such as orange oil, methyl salicylate, and clove oil, were added to 100-ml. volumes of various concentrations (1-8%) of sucrose monomyristate, agitating and mixing thoroughly, followed by measurement of the amounts of oil solubilized. An aqueous 8% sucrose monomyristate solution was incapable of completely solubilizing any of the volatile oils which were used. On the other hand, Tween 80[2] in 8% concentration was capable of solubilizing these volatile oils in water. The HLB (hydrophilic-lipophilic balance) value of sucrose monomyristate, calculated according to the method of Griffin (32), is 11.7 while that of Tween 80 is 15.0.

Applewhite, *et al.* (33), reported that Tween 80 would solubilize phenobarbital in water. Sucrose monomyristate was tested for this capacity. The test was made by adding phenobarbital in 0.1-Gm. amounts to 5% aqueous solutions of Tween 80 and sucrose monomyristate to the point of saturation of each solution. For six hours after the addition of the last portions of phenobarbital to these solutions they were maintained at 25° and stirred mechanically. The 100 ml. of 5% Tween 80 solution dissolved more than 0.5 Gm. but less than 0.6 Gm. of phenobarbital. The 100 ml. of 5% sucrose monomyristate dissolved more than 0.3 Gm. but less than 0.4 Gm. of phenobarbital. Phenobarbital is soluble normally to the extent of 0.1 Gm. in 100 ml. of water.

Emulsification Tests.—The "required HLB value" for mineral oil has been reported to be 10.5 (34), and 12 (35). Sucrose monomyristate, with HLB value of 11.7, was tested for its capacity to emulsify liquid petrolatum. Excellent emulsions were obtained using as little as 0.4% of sucrose monomyristate. A typical formulation was as follows:

Sucrose monomyristate	2.5 Gm.
Water	80.0 ml.
Methyl salicylate	0.2 ml.
Liquid petrolatum (heavy)	100.0 ml.
Sucrose	20.0 Gm.
Water	200.0 ml.
Liquid petrolatum (heavy)	200.0 ml.

A primary emulsion was prepared by dissolving the sucrose monomyristate in 80 ml. of water, dispersing therein the methyl salicylate by mechanical stirring, adding 100 ml. of liquid petrolatum while stirring vigorously, and finally dissolving the sugar in the mixture which was then forced through a hand homogenizer.[3] The remaining quantities of water and liquid petrolatum were added alternately in small amounts to the primary emulsion while stirring vigorously. The resulting mixture was then forced through the hand homogenizer, obtaining thereby an excellent, very palatable, and quite fluid emulsion.

The use of sucrose monomyristate as the sole emulsifying agent for vegetable oils, such as sesame, peanut, and cottonseed oil, produced less stable emulsions than those obtained when some sucrose dipalmitate was included in the formulation. Sucrose dipalmitate, being more lipophilic than the monoester, has an HLB value of 7.5. It is, therefore, a poor o/w emulsifier when used singly. It was found, for example, that an emulsion of cottonseed oil, capable of being autoclaved without destruction of the emulsion, could be prepared using a mixture of sucrose monomyristate and Sucrodet D-600[4] (sucrose dipalmitate) as the emulsifier. An emulsion for parenteral use would need to be sterilized. The following formulation yielded an emulsion, capable of being autoclaved, which might be useful in parenteral alimentation:

Sucrose monomyristate	0.85 Gm.
Dextrose	9.20 Gm.
Water	170.00 ml.
Sucrodet D-600	0.15 Gm.
Cottonseed oil	30.00 ml.

The sucrose monomyristate and dextrose were dissolved in the water, heating and stirring until solution was complete. The Sucrodet D-600 was similarly dissolved in the cottonseed oil. The hot (90°) oil solution was added slowly to the hot (90°) aqueous phase, stirring the mixture vigorously during the addition of the oil and for several minutes afterward. When slightly cooled, the pH of the mixture was adjusted to 7 with sodium hydroxide solution and then forced through the hand homogenizer, packaged, and autoclaved at 15-lb. pressure for twenty minutes.

Gelled Vehicle Preparation.—The sucrose diesters are not water soluble. It was found that an excellent dispersion of Sucrodet D-600 could be obtained in a syrup base. This dispersion was obtained by incorporating a hot (70°), alcoholic solution of the diester into hot (70°) syrup U. S. P., stirring until the mixture cooled to 40° or less. When the diester content was about 5%, the product obtained had an ointment-like consistency. It was gel-like since it had no noticeable tendency to flow. For purpose of identification in this section such "gelled" products are called syrup-diester dispersions.

By varying the concentration of the diester, the consistency of the syrup-diester dispersion may be controlled. The composition and consistency of these syrup-diester dispersions suggested their possible usefulness as pharmaceutical vehicles. These dispersions may be colored and flavored to produce very pleasing vehicles. Such vehicles prepared with syrup U. S. P., when exposed to the air, had a marked tendency to form a crystalline layer on the surface. The replacement of a portion of the syrup U. S. P. with Sorbo[5] (sorbitol solution, N. F.) eliminated this problem. The basic, unflavored formulation which was developed was as follows:

Sucrodet D-600	10 Gm.
Alcohol	10 ml.
Sorbo	50 ml.
Syrup U. S. P.	150 ml.

The Sucrodet D-600 and the alcohol were mixed

[2] Trademark of Atlas Powder Co., Wilmington, Del.
[3] Arthur H. Thomas Co., Philadelphia, Pa., No. 4308-S.
[4] Trademark of Millmaster Chemical Corp., New York, N. Y.
[5] Trademark of Atlas Powder Co., Wilmington, Del.

and heated to near boiling and added with vigorous stirring to a hot (70°) mixture of the Sorbo and syrup U. S. P., mixing until cool (below 40°).

Volatile oil flavors may be incorporated into these gelled vehicles by adding them to the alcohol-Sucrodet D-600 solution. If a flavored syrup is used, it may replace an equivalent volume of syrup U. S. P. For fruit-flavored products, the inclusion of some tartaric or citric acid is desirable. The gelled vehicle is adaptable to many flavor variations.

A variety of flavored gelled vehicles have been prepared. Some typical formulations appear in Table I.

TABLE I.—FLAVORED GELLED VEHICLE FORMULATIONS

Ingredients	Formulation 1	2	3	4	5
Sucrodet D-600, Gm.	10	10	10	10	10
Alcohol, ml.	10	10	10	10	10
Cherry syrup, ml.	50	...	...	...	...
Raspberry syrup, ml.	...	50	...	...	...
Chocolate syrup, ml.	...	...	50	...	...
Tartaric acid, Gm.	...	1	...	...	...
Citric acid, Gm.	...	...	...	...	1
Lemon oil	...	...	...	...	*q. s.*
Vanillin	...	...	...	*q. s.*	...
Color	*q. s.*	*q. s.*	*q. s.*	*q. s.*	*q. s.*
Sorbo, ml.	50	50	50	50	50
Syrup U. S. P., ml.	100	100	100	150	150

Microbiological Tests.—The fact that the sucrose esters contain a carbohydrate material suggested that preparations containing these esters might be prone to microbiological attack. The growth response of a number of frequently encountered microbiological contaminants was determined in media containing 0.5% and 1% sucrose monomyristate. Both of these media were tested singly and with an added nitrogen source appropriate for the organism being tested. No attempt was made to assure sterility of these media, since autoclaving of the ester solutions might have hydrolyzed a portion of the material. Effort was made to minimize contamination by sterilizing all equipment used, as well as the distilled water, and by carrying out all operations with the usual aseptic precautions observed in good bacteriological technique. The chemicals which were added to the sterile water in preparing the media were not sterilized. All media had pH values between 5 and 6. The inoculum in each instance, except for *Proteus vulgaris*, consisted of a suspension made by adding 8 ml. of sterile water to a thirty-six-hour agar-slant culture of the appropriate organism and then mixing the surface growth into the water as uniformly as possible. The inoculum for *Proteus vulgaris* was a thirty-six-hour nutrient broth culture of the organism. Besides *Proteus vulgaris*, the other organisms tested for growth response were *Saccharomyces cerevisiae, Aspergillus niger, Bacillus subtilis, Escherichia coli,* and *Pseudomonas fluorescens*. The volume of inoculum added to each 10-ml. sample of medium was 0.5 ml. The inoculated samples were incubated for six days at a temperature appropriate for the organism being tested. The results of these tests are summarized in Table II. The four yeast-inoculated cultures shown in Table II as having exhibited no growth at the end of six days were separately subcultured in malt extract broth. These subcultures were then incubated at 27° for six days. At the end of this incubation period, there was substantial growth present in each subculture, indicating that the yeast organisms had survived in the presence of low surface tension during the initial six-day incubation period.

TABLE II.—SIX-DAY MICROBIAL GROWTH RESPONSE IN SUCROSE MONOMYRISTATE SOLUTIONS

Organism	Nitrogen Source	Sucrose Monomyristate Solution —0.5%— Test	—0.5%— Control	—1.0%— Test	—1.0%— Control
Saccharomyces cerevisiae incubated at 27°	None	−[a]	−	−	−
	HN_4Cl 0.1%, K_2HPO_4 0.1%	−	−	−	−
Proteus vulgaris incubated at 37°	None	−	−	−	−
	Peptone 0.5%	+[b]	−	+	−
Aspergillus niger incubated at 27°	None	−	−	−	−
	HN_4Cl 0.1%, K_2HPO_4 0.1%	−	−	−	−
Bacillus subtilis incubated at 37°	None	+	−	+	−
	Peptone 0.5%	+	−	+	−
Escherichia coli incubated at 37°	None	−	−	−	−
	$(NH_4)_2SO_4$ 0.1%	−	−	−	−
Pseudomonas fluorescens incubated at 27°	None	+	−	+	−
	Peptone 0.5%	+	−	+	−

[a] No growth (−). [b] Growth (+).

Toxicity Tests.—Conflicting reports (1, 26, 27), without supporting experimental data, exist relative to the parenteral toxicity of the sucrose monoesters. For sucrose monomyristate, the intraperitoneal acute toxicity determination was made using the rat as the test animal. The animals used were five-week-old, Holtzman strain, first generation rats raised at the University of Nebraska. They had been numbered and sex separated at three weeks of age. Food was witheld for twenty-four hours prior to use; water was allowed *ad libitum*. A constant dose-volume of 10 ml./Kg. was injected intraperitoneally. Nine dosage levels were used, ranging from 100 mg. to 1,662 mg./Kg. All solutions injected were made isotonic by addition of sodium chloride as required. Five males and five females were injected at each dosage level, except No. 9 at which level five adult, unfasted, female Holtzman strain rats were used. After injection of the dose, measured to the nearest 0.01 ml., each rat was individually caged in a room maintained at 26–27°, deprived of water, and observed for twenty-four hours. The results from the injections are found in Table III.

The animals in the first four groups, or dosage levels, shown in Table III, exhibited no apparent evidence of toxicity. At dosage level No. 5, the animals became somewhat sedated; at No. 6 they became lethargic. The animals in groups 7, 8, and 9, although lethargic, evidenced considerable discomfort. Shortly prior to death, pronounced cyanosis of the feet, ears, and oral cavity developed.

TABLE III.—SUMMARY OF MORTALITY OF RATS TO INTRAPERITONEAL INJECTION OF SUCROSE MONOMYRISTATE

Group No.	Dosage level, mg./Kg.	No. of Rats	Elapsed Time Until death, hr. Min.	Max.	Mortality, %
1	100.0	10	..	..	0
2	142.1	10	..	..	0
3	201.9	10	..	..	0
4	286.9	10	..	..	0
5	407.7	10	..	..	0
6	579.3	10	3.03	21.07	50—LD_{50}
7	823.1	10	1.87	2.30	100
8	1117.1	9	1.56	1.93	100
9	1662.0	5	2.13	2.25	100

Death was attributed to anoxia with respiratory paralysis, as the heart continued to beat for a short time after respiration ceased. An autopsy of the expired animals revealed the presence of 2–5 ml. of pink-tinted to blood-tinted serous exudate in the abdominal cavity. The lungs were darker than normal with small hemorrhagic spots on the external surface. The blood vessels of the viscera were filled with dark, chocolate-colored blood. All other tissues and organs appeared to be essentially normal. One rat in group No. 6 succumbing twenty-one hours after injection evidenced a sloughing of the peritoneum. One rat in group No. 8 survived the entire test period, appearing to be quite normal. After twenty-four hours, the animal was sacrificed and upon autopsy appeared normal in every way except for some small hemorrhagic spots on the surface of the lungs. Injection into this rat must have been made into the lumen of the intestine. This animal was, therefore, discarded from the data.

Sucrose monomyristate was tested *in vitro* for its ability to hemolyze rat red blood corpuscles. About 2 ml. of blood were drawn by intracardial puncture from an etherized female rat randomly selected from the unused supply of rats remaining after the intraperitoneal toxicity study. One hundred units of heparin sodium injection U. S. P. were added to this blood. A sample of this heparinized blood was placed on a microscope slide and observed through a microscope as a small amount of 1% solution of sucrose monomyristate, previously made isotonic by the addition of sodium chloride, was brought in contact with the blood. Hemolysis of erythrocytes occurred promptly. When a drop or two of the 1% solution of sucrose monomyristate was added to about 1 ml. of the heparinized blood, the mixture was converted quickly into a clear, cherry-red solution, indicating hemolysis.

DISCUSSION

In the synthesis of sucrose monoesters by the procedure followed in this study, it has been postulated (15) that the methyl ester of the fatty acid reacts quite rapidly with sucrose to form diesters. These diesters, in the presence of a large excess of sucrose, react with the sucrose to yield essentially pure monoester material, with the 6-position of the glucose moiety of sucrose being esterified. This reaction mechanism was the basis of the unsuccessful alternate synthesis. The reasons for this failure are not understood. The conversion of preformed diester and sucrose into monoester should be, presumably, little different from the conversion of diester into monoester in the reaction sequence involving the methyl ester and sucrose.

Although the sucrose monoesters have been reported (26, 27) as being useful as solubilizers of vitamins A and D, their general usefulness as solubilizers appears to be quite limited. Sucrose monomyristate was disappointing in its solubilizing ability. It behaved, however, in accordance with its calculated HLB value.

In contradistinction to the success achieved in this laboratory with these sucrose esters as emulsifying agents, some workers have reported (36, 37) unsatisfactory results in attempts to use sucrose monoesters as emulsifying agents for lipid materials. In view of the results of the acute intraperitoneal toxicity test, the intravenous administration of emulsions containing sucrose monomyristate should be approached with caution.

The gelled vehicles appear to be very suitable for the incorporation of powdered materials. These vehicles can be suitably flavored to meet a variety of demands. This dosage form may be packaged in collapsible tubes and could prove to be a convenience to parents in administering medication to children. In addition, the problem of spilled medicines on the bed linens, etc., should be minimized with such a gelled dosage form. These gelled vehicles developed from Sucrodet D-600 are seemingly quite versatile and could have a rather wide range of applications.

The microbiological tests indicate that no unusual or extraordinary precautions against microbial contamination are necessary because of the inclusion of sucrose monomyristate in aqueous preparations or formulations. Spoilage, due to microbial growth, of such preparations was not observed. No study was made relative to the problem of preserving any of these preparations. Although surfactants depending upon a polyoxyethylene grouping for water solubility have been reported (38–43) as inactivating phenolic types of preservatives, it has been asserted (28, 44) that the sucrose esters do not inactivate phenolic preservatives. One study (45), however, appears to contradict this conclusion. The matter of selecting suitable preservatives to use with the sucrose esters remains unsettled.

The sucrose monomyristate solutions which were injected intraperitoneally into rats contained, at the lowest toxic concentration, 10 to 20 times more monoester than did the emulsions prepared for parenteral use. The intravenous administration of a surfactant as a component of an emulsion system might be less toxic than when administered singly in identical concentration, due to the fact that in the emulsion the surfactant is part of a somewhat fixed system. Thus, the surfactant in an emulsion might not be instantly available to produce toxic effects. This question was not investigated. However, the intravenous toxicity of sucrose monomyristate should be determined by an animal study.

SUMMARY

1. An improved procedure for preparing the sucrose mono-fatty acid esters has been developed. This process eliminates bitter-tasting substances present in the final product pre-

pared by the previously recommended procedure.

2. Sucrose monomyristate has been found to be a poor solubilizer for essential oils, thereby reducing its potential usefulness in pharmaceutical formulation.

3. The sucrose esters used in this study are good emulsifying agents. Mixtures of the monoester and diester were more suitable for emulsifying vegetable oils than either of the esters when used singly. The mixtures may be more effective due to better approximation of the required HLB of the oils. Sucrose monomyristate produced excellent liquid petrolatum emulsions.

4. The calculated HLB values for the sucrose esters used in this study appear to be in agreement with experimental findings of this study.

5. Sucrose dipalmitate, Sucrodet D-600, in a syrup base produced an excellent semisolid, gelled vehicle. This vehicle may be flavored as desired. These flavored, gel-like vehicles are recommended as being well-suited to pediatric use.

6. The sucrose esters used in this study do not appear to favor microbial growth.

7. Intraperitoneal acute toxicity was determined for sucrose monomyristate using the rat. The LD_{50} was 579 mg./Kg. Sucrose monomyristate produced rapid hemolysis of rat blood *in vitro* at low concentrations.

8. Although the sucrose fatty acid esters may have what appears to be a rather limited range of usefulness due to their inability to solubilize certain materials effectively, nevertheless, they do appear to be well-suited for use as emulsifying agents and for the preparation of inexpensive, flavorable, gel-like vehicles which are suitable for use with many therapeutic agents.

9. These new, surface-active materials appear to possess sufficient pharmaceutical potential to warrant a complete toxicological study.

REFERENCES

(1) Hass, H. B., *Chemurgic Dig.*, **14,** No. 6, 8(1955).
(2) Anon., *Mfg. Chemist*, **24,** 368(1953).
(3) Anon., *Chem. Week*, **75,** Sept. 11, 49(1954).
(4) Anon., *ibid.*, **77,** Oct. 8, 90(1955).
(5) Pacini, A. B., *Drug and Allied Inds.*, **41,** Dec., 12 (1955).
(6) Anon., *Drug & Cosmetic Ind.*, **78,** 239(1956).
(7) Hass, H. B., *Chem. in Can.*, **8,** April, 39(1956).
(8) Anon., *Drug and Allied Inds.*, **42,** Dec., 15(1956).
(9) Osipow, L., *J. Soc. Cosmetic Chemists*, **7,** 249(1956).
(10) Pacini, A. B., *Drug and Allied Inds.*, **43,** May, 4(1957).
(11) Anon., *Dodge & Olcott News*, **10,** Aug., 4(1957).
(12) Ancona, A., *Boll. chim. farm.*, **97,** 401(1958).
(13) Osipow, L., Marra, D., and Snell, F. D., *Drug & Cosmetic Ind.*, **80,** 312(1957).
(14) Hass, H. B., *Mfg. Chem.*, **29,** 152(1958).
(15) Osipow, L., Snell, F. D., York, W. C., and Finchler, A., *Ind. Eng. Chem.*, **48,** 1459(1956).
(16) Anon., *Drug & Cosmetic Ind.*, **79,** 824(1956).
(17) Anon., *Ind. Eng. Chem.*, **48,** Oct., 13A(1956).
(18) Anon., *Chem. Eng. News*, **35,** April 29, 108(1957).
(19) Anon., *ibid.*, **35,** May 27, 80(1957).
(20) Anon., *ibid.*, **35,** July 8, 78(1957).
(21) Anon., *ibid.*, **36,** April 21, 24(1958).
(22) York, W. C., Finchler, A., Osipow, L., and Snell, F. D., *Intern. Congr. Pure and Appl. Chem.; XIV Congr.*, Zurich, Switzerland, July 21–27, 1955; through *Ind. Eng. Chem.*, **48,** 1459(1956).
(23) York, W. C., Finchler, A., Osipow, L., and Snell, F. D., *J. Am. Oil Chemists' Soc.*, **33,** 424(1956).
(24) Anon., "Sucrodets: General Information Brochure," and "Sucrodet D-600: Technical Data Sheet," Millmaster Chemical Corp., 295 Madison Avenue, New York 17, N. Y., August 1957.
(25) Osipow, L., Snell, F. D., Marra, D., and York, W. C., *Ind. Eng. Chem.*, **48,** 1462(1956).
(26) Mima, H., *Pharm. Bull. (Tokyo)*, **5,** 496(1957).
(27) Mima, H., *Yakugaku Zasshi*, **78,** 988(1958); through *Chem. Abstr.*, **53,** 1636(1959).
(28) Raphael, L., *Mfg. Chem.*, **29,** 237(1958).
(29) Fitzhugh, O. G., and Nelson, A. A., THIS JOURNAL, **37,** 29(1948).
(30) Lambert, G. F., Miller, J. P., and Frost, D. V., *ibid.*, **45,** 685(1956).
(31) Raphael, L., *Mfg. Chem.*, **29,** 105(1958).
(32) Griffin, W. C., *J. Soc. Cosmetic Chemists*, **5,** 249 (1954).
(33) Applewhite, R. W., Buckley, A. P., and Nobles, W. L., *J. Am. Pharm. Assoc., Pract. Pharm. Ed.*, **15,** 164(1954).
(34) Anon., "Atlas Surface Active Agents," Atlas Powder Co., Wilmington, Del., 1950, p. 28.
(35) Anon., "Guide to the Use of Atlas Surfactants and Sorbitol in Pharmaceutical Products," Atlas Powder Co., Wilmington, Del., 1958, p. 22.
(36) Tober, T. W., and Autian, J., *J. Am. Pharm. Assoc., Pract. Pharm. Ed.*, **19,** 422(1958).
(37) Hom, F. S., "The Preparation and Stabilization Studies of Ethiodol Emulsions for Intravenous Hepatolienography," Thesis, Temple University, Philadelphia, Pa., 1956, pp. 16–20.
(38) deNavarre, M. G., *Congr. mondial detergence et prods. tensio actifs, Ier Congr., Paris*, **2,** 741(1954); through *Chem. Abstr.*, **51,** 14294(1957).
(39) deNavarre, M. G., and Bailey, H. E., *J. Soc. Cosmetic Chemists*, **7,** 427(1956); through *Chem. Abstr.*, **51,** 1549 (1957).
(40) Hall, C. S., and deNavarre, M. G., *Proc. Sci. Sect. Toilet Goods Assoc.*, **27,** 29(1957); through *Chem. Abstr.*, **51,** 11659(1957).
(41) Poprzan, J., and deNavarre, M. G., *J. Soc. Cosmetic Chemists*, **10,** 81(1959).
(42) Mulley, B. A., and Metcalf, A. D., *J. Pharm. and Pharmacol.*, **8,** 774(1956).
(43) Barr, M., and Tice, L. F., THIS JOURNAL, **46,** 445 (1957).
(44) Moore, C. D., and Hardwick, R. B., *Mfg. Chem.*, **29,** 194(1958).
(45) Wedderburn, D. L., *J. Soc. Cosmetic Chemists*, **9,** 210 (1958).

The Effects of Tranquilizers on Bacterial Toxemias I. Reserpine*

By LEO GREENBERG, JAMES W. INGALLS, and ARTHUR G. ZUPKO

Purified exotoxins of *Clostridium tetani* and *Clostridium botulinum* are highly lethal to mice. Pretreatment of experimental animals with a single, large dose of reserpine prior to toxin administration resulted in a highly significant prolongation of survival time. Similar prolongation was achieved in overwhelming infection induced with *Diplococcus pneumoniae*. This effect was not demonstrated with small doses of reserpine administered in a divided series either prior to or concurrent with the infection.

It is a widely accepted concept today that bacterial infection places a stress upon the diseased animal. In view of the potent pharmacological activity of the tranquilizers in ameliorating many of the stress factors of modern living, it seemed of interest to investigate what effects, if any, these drugs might have on disease processes of bacterial origin. Such studies have now been under way at our laboratories since 1957, and the present paper deals with the role of reserpine in some bacterial stress situations.

Although studies of reserpine action have been largely confined to the area of emotional or psychological stress, several references to its role in physiological stress have appeared in the literature. Thus, it is known that the drug is of value in protecting animals against hemorrhagic shock (1), while in experimental *Staphylococcus aureus* infection it may exacerbate the infectious process through the depletion of 5-hydroxytryptamine (2). It has also been reported that in a dose of 5 mcg., reserpine increased the toxicity of *Neisseria gonorrhoeae* endotoxin for mice, while at a dose of 2 mcg., no significant effects were observed (3). On the other hand, a significant prolongation of survival time from *Clostridium botulinum* toxin with 10 mcg. reserpine has been noted in mice (4).

MATERIALS AND METHODS

Male mice weighing approximately 25 Gm. were used throughout these experiments. Preliminary experiments with divided doses of reserpine and the pneumococcal studies were done with CFW and Rockefeller strain animals. All other data were derived from CF1 (Carworth Farms) animals. Mice were caged in small groups and kept in the thermostatically-controlled animal house for several days prior to use.

Reserpine solution[1] was diluted with distilled water and all inoculations were made by intraperitoneal injection. Except where otherwise indicated, the drug was injected approximately forty-five minutes prior to introduction of the toxic agent.

Tetanus toxin[2] in 0.85 NaCl solution with 0.3 *M* glycine, an Lf. titer of 870/ml., and a mouse LD_{50} of 112 million was used throughout. Purified *Clostridium botulinum* type A Toxin[3] diluted with two parts of glycerol and a mouse LD_{50} of approximately 5×10^6/ml. was used. Toxins were stored in the refrigerator, the botulinus material at $-20°$ to maintain potency. All dilutions were made with sterile isotonic saline. Toxin injections were always in a volume of 0.1 ml. and were administered intraperitoneally.

Diplococcus pneumoniae strain SV1 was obtained as a lyophilized culture from the Rockefeller Institute and grown in brain-heart infusion broth (Difco). Slants were maintained on brain heart infusion agar with added blood. Subculturing was done weekly and virulence maintained by monthly mouse passage. For inoculations, an eighteen-hour broth culture containing approximately 2×10^8 organisms was used, injections being by the intraperitoneal route.

In all experiments, paired groups of animals of the same weight, age, and source were used and all control and experimental animals in any given run were inoculated within a few minutes of one another. None of the diluent substances used showed any toxicity to mice, and sterile brain heart infusion broth injections were shown to be without effect.

RESULTS AND DISCUSSION

Toxin Studies.—A highly significant prolongation of life in both tetanus and botulinus toxemia can be seen from the data in Table I. It will be noted, however, that the combination of agents used is a specific one—a large dose of the tranquilizer and an overwhelmingly lethal dose of the toxin. This combination was determined after preliminary experiments with tetanus toxin involving more than 600 mice indicated that this approach was the most fruitful, yielding consistently reproducible results. Other approaches investigated included:

Use of Highly Dilute Toxin.—In control animals it was determined that 50% of the animals injected with various dilutions of toxin died as follows:

* Received August 21, 1959, from the Research Institute of the Brooklyn College of Pharmacy, Long Island University, Brooklyn 16, N. Y.

Supported in part by a grant from Wallace Laboratories, New Brunswick, N. J.

Presented to the Scientific Section, A. PH. A., Cincinnati Meeting, August 1959.

[1] Supplied as Serpasil through the courtesy of Ciba Pharmaceutical Products, Inc., Summit, N. J.

[2] Supplied through the courtesy of Dr. H. A. Dettwiler, Eli Lilly & Co., Indianapolis, Ind.

[3] Supplied through the courtesy of Matteo Cardella, Immunology Branch, U. S. Army Biological Warfare Laboratories, Fort Detrick, Frederick, Md.

TABLE I.—EFFECT OF RESERPINE ON SURVIVAL TIME FROM TETANUS AND BOTULINUS TOXINS

Treatment				
Toxin 0.1 ml., 1:100	Reserpine mg./Kg.	No. of Animals	Mean Survival Time, min.	Significance
Botulinus	0	10	129.3 ± 6.1	Difference between control and both experimental groups is highly significant, P <0.001
Botulinus	2.0	10	163.4 ± 9.8	
Botulinus	2.0	10	161.4 ± 8.6	
Tetanus	0	80	151.0 ± 7	
Tetanus	0.2	10	183.7 ± 39	Difference not significant, P >0.4
Tetanus	2.0	10	275.3 ± 43	Difference is significant, P <0.02
Tetanus	0	20	108.1 ± 5.5	
Tetanus	2.0	10	226.3 ± 16	Differences are both highly significant, P <0.001
Tetanus	3.0	10	224.2 ± 17	

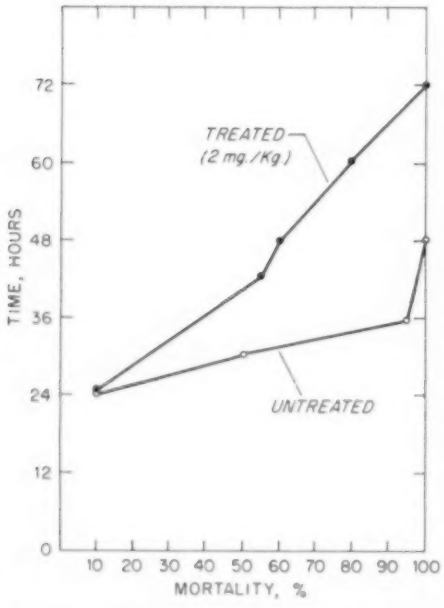


Fig. 1.—Effect of reserpine on survival time from pneumococcal bacteremia.

1:50,000/twenty-four hours; 1:100,000/thirty hours; 1:150,000/thirty-six hours; 1:200,000/forty-four hours; 1:250,000/seventy-two hours; 1:300,000/one hundred and twenty hours. A single dose of 0.1 mg./Kg. reserpine failed to show any significant departure from these survival figures.

Pretreatment with Repeated Reserpine Injections.—In 50 animals injected with 1:400,000 toxin, 54% were dead within twelve hours. Daily injections of 0.1 mg./Kg. reserpine for five days prior to toxin administration resulted in an apparent increased lethality of the toxin, 80% of the animals being dead within twelve hours. Likewise, when three injections of 0.1 mg./Kg. reserpine were given eight hours apart for twenty-four hours prior to toxin introduction, no significant changes in survival time were found in groups of ten animals each at toxin dilutions of 1:2,000; 1:5,000; 1:10,000; 1:20,000; 1:50,000; 1:100,000; 1:150,000; 1:200,000; 1:250,000; 1:300,000.

Use of Repeated Post-toxin Reserpine Injections.—Groups of ten animals were given toxin dilutions of 1:200,000; 1:300,000; 1:400,000, and 1:500,000. Reserpine at 0.1 mg./Kg. was given immediately thereafter and once daily until the death of the animal. At all levels, a trend toward increased survival was noted, but results were without statistical significance.

Pneumococcal Studies.—As in the case of the toxin studies previously outlined, the combination of a large dose of reserpine administered approximately forty-five minutes prior to the introduction of a toxic agent of overwhelming lethality, in this case an undiluted broth culture of virulent pneumococci, resulted in a highly significant prolongation of survival time. A typical response is shown in Fig. 1.

Use of undiluted broth culture was decided upon after ten weeks of preliminary testing. It was found that the limits of survival time of mice could be more sharply defined when undiluted culture was used than when any dilution of the culture was employed. Dilution of the culture in sterile broth or saline prolonged survival time compared to undiluted culture, but did not prolong it reliably in proportion to the dilution.

In 29 control groups of ten mice, each injected with 0.1 ml. of undiluted broth culture of pneumococci, 90 to 100% of the animals died between the sixteenth and forty-eighth hour. Pretreatment with 0.2 mg./Kg. reserpine, either prior to or subsequent to infection, revealed no gross deviation in survival patterns. When the dose of reserpine was increased to 2.0 mg./Kg. or more, significant prolongations of survival time could be demonstrated repeatedly, and apparently the extent of such prolongation is directly proportional to the dose employed, being limited at its upper limits by the toxicity of the reserpine itself.

SUMMARY AND CONCLUSIONS

1. Groups of mice were injected with overwhelmingly lethal doses of three toxic agents, *Clostridium tetani* exotoxin, *Clostridium botulinum* exotoxin, and *Diplococcus pneumoniae* broth culture.

2. Pretreatment forty-five minutes prior to the introduction of the toxic agent with a single dose of reserpine, 2 mg./Kg. or higher, resulted in highly significant prolongation of survival time.

3. Neither post-toxin treatment with reserpine

nor pretreatment with small divided doses evidenced this effect in our experiments.

4. In addition to its proved usefulness in emotional and psychological stress, reserpine is apparently capable of favorably influencing the course of bacterial stress in laboratory animals.

REFERENCES

(1) Fine, J., Frank, E. D., Ravin, H. A., Rutenberg, S. H., and Schweinburg, F. B., *New Engl. J. Med.*, **260**, 214(1959).
(2) Mishra, B. P., and Sanyal, R. K., *J. Pharm. and Pharmacol.*, **11**, 127(1959).
(3) Tauber, H., and Garson, W., *Proc. Soc. Exptl. Biol. Med.*, **97**, 886(1958).
(4) Boroff, D. A., *Intern. Arch. Allergy Appl. Immunol.*, **15**, 74(1959).

Binding of Drugs by Plastics II*

Interaction of Weak Organic Acids With Plastic Syringes

By H. K. KIM and J. AUTIAN

Three plastic syringes were evaluated as to their ability to bind a number of weak organic acids. Polyethylene and polystyrene syringes did not interact with any of the seven agents included in the binding study. Benzoic acid, *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, and salicylic acid were bound to various degrees by the nylon syringes. No interaction was noted with any of the syringes and acetylsalicylic acid, phenobarbital, or barbital. The degree of binding was influenced by concentration, temperature, and diffusion.

THE RAPID INCREASE in the use of plastics in place of glass, rubber, and metals is very evident by observing the multitude of products now composed, in whole or part, of plastic. Today, in many instances, a plastic may be formulated to convey certain outstanding properties to a product while minimizing objectionable properties. The success of these plastic materials in practically all fields has created in the minds of many a confidence in plastics which is a tribute to the scientific achievements of the plastic industry.

It is rather unfortunate that this "confidence" is now engendered by certain groups in both the medical and pharmaceutical field. Since plastics is still relatively in its infancy in medical and pharmaceutical practice, it is not too surprising to see or hear of instances where plastic materials have been utilized without proper evaluation first taking place. Usually, there is no problem when an ethical pharmaceutical firm utilizes a plastic material for a container, syringe, or tubing, since extensive tests are conducted to insure that the plastic material has no unusual effect upon the specific drug system to be used in the plastic device. Some concern, however, should be elicited by those clinicians or pharmacists who are, or will be utilizing plastic devices for various medicinal products, i. e., syringes, tubings, etc. In this respect, Bryant and Brewer (1) have suggested that new toxicity and safety tests should be initiated when disposable pharmaceutical materials are to be employed.

Recently reports have appeared which point out some of the difficulties encountered in the use of plastic devices with certain medicinal preparations.

Autian and Brewer (2) noted in their study of plastic-hubbed needles that a certain plastic formulation released a toxic constituent to saline solution. They further found that a solvent (diethyl carbonate) in a particular parenteral product dissolved the plastic hubs.

In the evaluation of three types of plastic syringes (polyethylene, polystyrene, and nylon) as to their physical compatibility with parenteral products, a number of incompatibilities were seen with the polystyrene but not with the other two plastic materials (3).

Certain plastic tubings to be used as medical grade tubing were found to leach acidic constituents to drug solutions (4). It was rather interesting to note that in certain solutions heavy leaching occurred; whereas in others only minor or no leaching occurred.

Still another problem has been encountered with the use of plastics in syringes. In this case, Marcus, *et al.* (5), reported that nylon syringes bound to various degrees, a number of bacteriostatic agents. They found that parahydroxybenzoic acid, methylparaben, propylparaben, sorbic acid, phenol, and 4-chloro-3-methylphenol were bound by the nylon.

* Received September 10, 1959, from the College of Pharmacy, University of Michigan, Ann Arbor.
Presented to Scientific Section, A. PH. A., Cincinnati meeting, August 1959.
This research project was conducted under a grant from the Becton, Dickinson and Co. Rutherford, N. J.

Even though the number of incompatibilities appear to be rather limited, the brief review above should indicate that incompatibilities may occur which cannot be easily detected by the clinician or nurse. This is especially true of those cases where plastic materials bind the drug and thus prevent the prescribed dose being administered to the patient.

This paper continues the study previously reported (5) on the binding of drugs by plastics. In this particular study a number of weak organic acids was included to determine if one or more of the drugs would be bound by the three types of plastic syringes.

EXPERIMENTAL

Apparatus and Reagents

Apparatus.—Beckman DU spectrophotometer; Beckman pH meter, model G; and constant temperature ovens.

Plastic Syringes.—Polyethylene, Becton, Dickinson and Co., Rutherford, N. J.; polystyrene, Becton, Dickinson and Co.; nylon, Atlas Surgical Supplies, Ltd., Manchester, England, distributed in U. S. by Child's Surgical Supply, Inc., Pasadena, Calif.

Reagents.—Benzoic acid, U. S. P., *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, N. F., salicylic acid, R. G., acetylsalicylic acid, U. S. P., phenobarbital U. S. P., barbital N. F.

Methods of Analysis

Benzoic Acid, *m*-Hydroxybenzoic Acid and Salicylic Acid.—A titration procedure was employed to determine the quantity of these acids. A standard barium hydroxide solution (0.05 *N*) was used as the titrant and phenolphthalein as the indicator.

Acetylsalicylic Acid.—The quantitative analysis of aspirin was conducted by employing a titration procedure using a standard alcoholic potassium hydroxide solution (0.02 *N*) as the titrant and α-naphtholbenzein as the indicator.

***p*-Aminobenzoic Acid.**—A spectrophotometric method was used to determine the absorbance of *p*-aminobenzoic acid at a wave length of 270 mμ and in a concentration of 1 mg./100 ml. Distilled water was employed as a blank.

Phenobarbital and Barbital.—Both drugs were assayed by a spectrophotometric method at a wave length of 240 mμ in a borate buffer system (pH 9.4). Borate buffer solution was used as the blank, and the concentrations employed for both barbiturates in the cell were adjusted to a strength of 1 mg./100 ml.

Binding as a Function of Concentration and Temperature

Two-milliliter size syringes were employed in this study with the needles removed and the tip at the junction of the canula and barrel fused by application of heat.

The binding experiments were essentially those reported in a previous paper (5). Four concentrations[1] for each agent were prepared using double-distilled water as the solvent. Each solution was then added to each barrel, stoppered with a cork encased in parafilm, and stored for a period of forty-eight hours at a specified temperature (5, 30, and 50° ± 1°). Three barrels were employed for each concentration and a control sample (in a Pyrex test tube).

After the storage period, the barrels were removed and the contents pooled for each concentration (and for each drug). The pooled samples were then assayed for their respective drugs and the results calculated as the average value for one barrel.

Neither the polyethylene nor the polystyrene barrels bound, to any degree, the drugs included in this study. Four of the seven drugs were bound, to various degrees, by the nylon barrels. These were benzoic acid, *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, and salicylic acid. Figures 1–4 relate the quantity of bound drug to the quantity unbound.

Acetylsalicylic acid and the two barbituric acids were not bound, to any extent, by the nylon barrels.

Binding as a Function of Time

Solutions were prepared of the eight drugs and placed into each type of plastic barrel, stoppered, and stored at 30°. Sufficient barrels were employed to insure that duplicate samples of each drug could be assayed over a period of one week.

As before, benzoic acid, *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, and salicylic acid were bound by the nylon barrels. Figure 5 contains the results for the week period (in hours) expressed as drug bound (in per cent). The nylon barrels did not

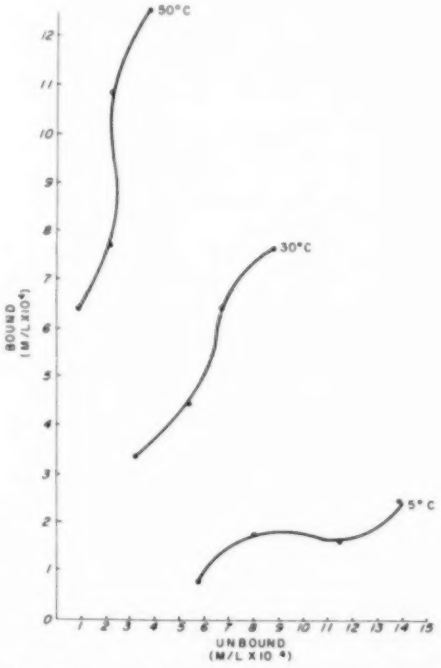


Fig. 1.—Binding of benzoic acid by nylon syringes.

[1] 0.001 *M* H_2SO_4 was used to suppress hydrolysis in the aspirin solution.

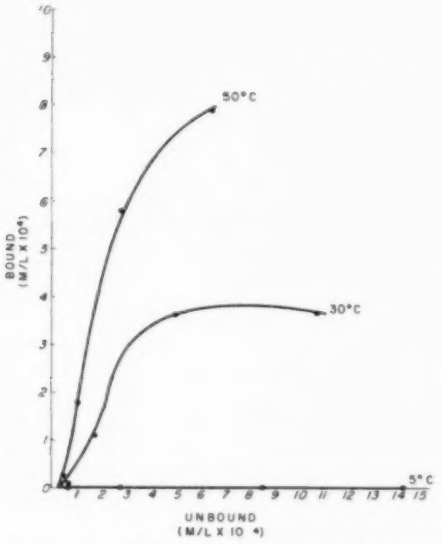


Fig. 2.—Binding of *m*-hydroxybenzoic acid by nylon syringes.

bind the other three drugs. None of the drugs were bound by either the polyethylene or polystyrene barrels.

DISCUSSION

Even though it was assumed that no binding would occur with either the polyethylene or polystyrene barrels because of the lack of polar centers in the macromolecules, these two types of barrels were included in the study to detect if other physical or chemical reactions would occur. As reported, no such reaction was observed for any of the drugs included in the experiments within the one-week period.

Previous investigation with nylon barrels (5) confirmed earlier thoughts that nylon syringes would bind or sorb certain drugs possessing acidic hydrogens. The carbonyl groups of the polyamide structure would be expected to attract those molecules possessing proton-donating groups such as phenols and weak organic acids. The energy required for binding appears to be primarily due to dipole-dipole interaction between the reactants; however, secondary attraction forces (van der Walls) must contribute sufficient energy to achieve a stable interaction (binding). It has been pointed out that dipole-dipole interaction alone would not produce stable binding, since very polar water molecules would also be competing with the acidic molecules for the binding sites in the polyamide (6). The relatively large concentration of water molecules to the acidic molecules would favor statistically the interaction of the water molecules to the negative sites in the polyamide.

As may be seen from Figs. 1–5, binding occurred with four drugs (benzoic acid, *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, and salicylic acid) of the seven weak organic acids. In each instance,

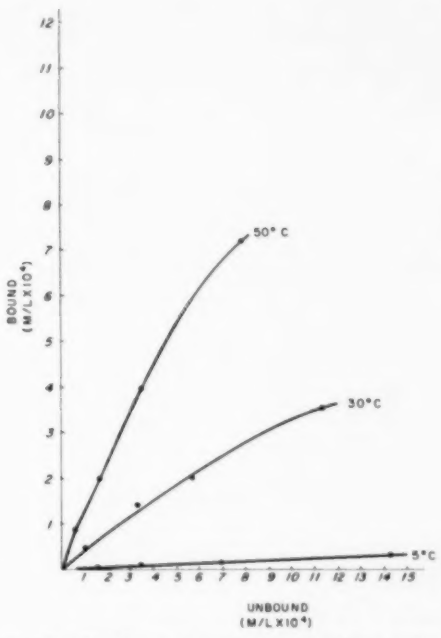


Fig. 3.—Binding of *p*-aminobenzoic acid by nylon syringes.

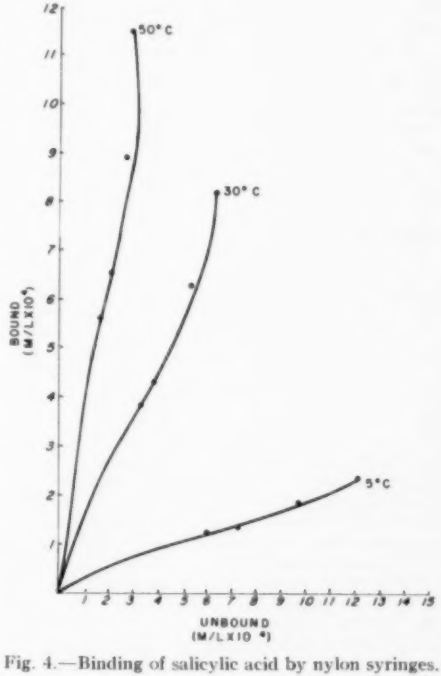


Fig. 4.—Binding of salicylic acid by nylon syringes.

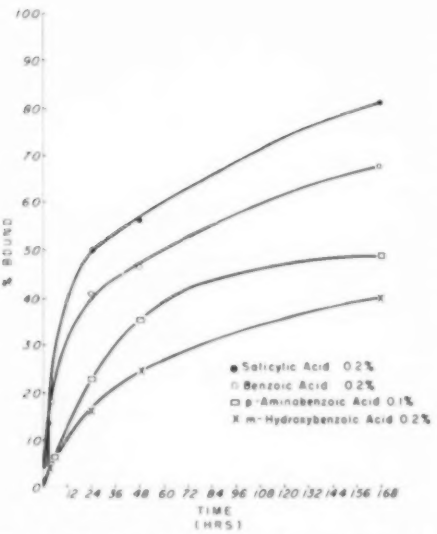


Fig. 5.—Binding of drugs by nylon syringes as a function of time.

concentration, temperature, and contact time were factors influencing the binding.

The magnitude of binding (quantity of drug or agent bound) shown in this paper and a previous paper indicates that diffusion is taking place, since the quantity of drug uptake cannot be attributed to surface binding alone. The number of sites on the surface exposed to the solution is relatively small compared to the quantity of molecules bound. It may be reasonably assumed that after the surface sites are filled, the solution permeates into the nylon where new sites for binding become available. Since no experiments were conducted to determine diffusion rates, quantitative interpretation of the diffusion phenomena could not be given. Certain results, however, gave strong evidence that the diffusion rates could be altered by the drug or agent, thus increasing or decreasing the total amount of drug bound.

In a previous report (5) the plot of bound agent *vs.* unbound agent resembled, to a first approximation, a Langmuir adsorption isotherm. Phenol and 4-chloro-3-methylphenol produced anomalous results which were explained on the basis that the two phenols were altering the structure of the macromolecule permitting a rapid surge of solution into the nylon when a certain critical concentration of the agent was reached. At the lower concentration range the two phenols mentioned above were taken up slowly until a critical concentration was reached where a dramatic uptake was noted.

In this study *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, and salicylic acid (at 5°) followed the Langmuir adsorption isotherm when plotted as mentioned previously (Figs. 2, 3, and 4).

The plot for benzoic acid (Fig. 1) produced an S-shaped curve for each of the three temperatures. No definite explanation could be given for this type of curve, since sufficient data were not available for exact interpretation. The curve shows that the uptake of the acid was not occurring in a smooth manner but rather in spurts as the concentration was increased. This type of curve suggests that in certain concentration ranges the benzoic acid had little difficulty reaching binding sites in the polymer. Once the polymer area in contact with the solution became saturated, no further binding could take place until the solution penetrated deeper into the plastic. When this diffusion took place a new series of sites became available for further binding.

The effect of temperature on binding is readily seen in Figs. 1–4, an increase in temperature causing greater binding. It is interesting to observe that within the forty-eight-hour period no binding occurred for *m*-hydroxybenzoic acid and very little binding for *p*-aminobenzoic acid at 5°. The increase in binding at the higher temperatures for all the drugs was assumed to be due to an increase in the diffusion rate of the solution into the plastic.

The one week's study (Fig. 5) indicates that significant quantities of the agents are bound even after short periods of time. Salicylic acid demonstrated the greatest degree of binding (Figs. 4 and 5) of the seven acidic agents included in the study. The esterification of the phenolic group in the salicylic acid (aspirin) obliterated this binding ability, probably because of the stereochemical effect.

The lack of binding of phenobarbital and barbital again might best be explained on steric hindrance preventing proper orientation of the acidic molecule to the sites in the macromolecule.

Results of the binding studies give further evidence that plastic materials should be thoroughly evaluated with the drug system to be employed or to be placed in contact with the plastic before routine use. The replacement of glass by a plastic material should necessitate careful considerations since glass has not shown the incompatibilities reported for plastic materials.

SUMMARY

1. A previous study was continued to determine if three types of plastic syringes would bind seven weak organic acids.

2. Polyethylene and polystyrene barrels did not bind any of the seven agents.

3. The nylon barrel bound to various degrees, four of the seven agents (benzoic acid, *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, and salicylic acid).

4. Acetylsalicylic acid, phenobarbital, and barbital were not bound by any of the plastic barrels.

5. The degree of binding was influenced by concentration, temperature, and diffusion.

REFERENCES

(1) Brewer, J. H., and Bryant, H. H., personal communication.
(2) Autian, J., and Brewer, J. H., *Am. J. Hosp. Pharm.*, **15**, 313(1958).
(3) Autian, J., and Dhorda, C. N., *ibid.*, **16**, 176(1959).
(4) Autian, J., *Bull. Parenteral Drug Assoc.*, **12**, 17(1958).
(5) Marcus, E., Kim, H. K., and Autian, J., THIS JOURNAL, **48**, 457(1959).
(6) Higuchi, T., and Lach, J. L., *ibid.*, **43**, 465(1954).

Studies on the Inhibitory Effect of Combined Chemical Preservatives on *Saccharomyces cerevisiae**

By H. G. OSMAN and AFAF EL-MARIAH

The minimal concentrations (Gm./L.) of formic, salicylic, benzoic, *o*- and *p*-chlorobenzoic acids, methyl-, ethyl-, propyl-, butyl esters of *p*-hydroxybenzoic acid, and sorbic acid required to inhibit the growth of *Saccharomyces cerevisiae* were determined. Combinations of any two of these chemicals were also tested for the same action. Salicylic acid with any of the other chemicals proved to have a potentizing effect. Other combinations had either an additive effect or even a lesser effect than their components used singly.

THE INHIBITORY EFFECT of many chemicals on the growth of microorganisms has been studied by many investigators. It was found that a chemical preservative may strongly inhibit the growth of certain microorganisms while having a lesser effect on others. Therefore, a mixture of two or more preservatives would have a wider scope of inhibition than their components when tested alone. This point was investigated by Sabalitschka (1), in 1932, who recommended the use of a mixture of 60 per cent ethyl *p*-hydroxybenzoate and 40 per cent propyl *p*-hydroxybenzoate for the protection of foodstuffs against deterioration through bacterial or fungal action.

Schelhorn (2), in 1950, noticed that in the presence of citric acid less benzoic acid was required to suppress the growth of microorganisms than when benzoic acid was used alone.

Littlejohn and Husa (3), in 1955, and Schimmel and Husa (4), in 1956, showed that there was either a definite potentizing action or an additive effect when mixtures of *p*-hydroxybenzoates were used as preservatives for syrups.

A further advantage of the combinations of *p*-hydroxybenzoic acid esters has been put forward by Boehm (5), in 1959, who found an increase in the solubilities of long-chain esters when they were combined together.

In this paper, some chemical preservatives were studied for their inhibitory effect on the growth of *Saccharomyces cerevisiae*. All possible combinations from two preservatives were prepared and their inhibitory effects were studied.

EXPERIMENTAL

The test organism used throughout all these investigations was *Saccharomyces cerevisiae* which was kindly offered by Dr. K. Raible of the Deutsche Forschungsanstalt für Lebensmittelchemie. All chemicals used were of the extra pure or A. R. Merck grade.

Saccharomyces cerevisiae was cultured on a semi-synthetic liquid medium containing, per L. (w/v); 30 Gm. sucrose; 8 Gm. citric acid; 3 Gm. $NH_4H_2PO_4$; 8.9 Gm. $Na_2HPO_4 \cdot 12H_2O$; 0.1 Gm. $CaCl_2$; 0.5 Gm. $MgSO_4 \cdot 7H_2O$; 0.1 Gm. KCl; 1.5 Gm. Difco peptone; and 1.5 Gm. Difco yeast extract. The pH of the culture medium was always adjusted to 4.

The microorganism was subcultured on slopes of the same culture medium containing % (w/v) 2.5 Gm. agar agar. Transference of the yeast cells was carried out every month.

Sterilization of either the liquid culture medium or the slopes was always done at 5 lb./sq. in. for thirty minutes. The culture tubes or flasks were inoculated with a forty-eight-hour old liquid inoculum. The volume of the inoculum was always 2%. The cultures were incubated at 30° for one week. Unless otherwise specified, all experiments were carried out in 20-ml. test tubes containing 5 ml. culture medium.

For the determination of the growth of the yeast cultures the method adopted by Goodwin and Osman (6), in 1953, was applied. The relation between the absorbance at 1,000 mμ and the dry weight of the yeast cells has been studied. It was found that there was a linear relationship between the dry weight contents of the yeast cells in the liquid culture media and their corresponding extinctions at 1 μ over a wide range of *E* values. Figure 1 illustrates this relationship.

The *E* values were measured at 1 μ in order to exclude any absorption of light which might be due to any coloration of the liquid medium. Actively growing cultures were diluted beforehand with the liquid culture medium in order to obtain suitable readings on the Zeiss spectrophotometer model PMQ II.

RESULTS AND DISCUSSION

In order to determine the period of incubation for the yeast cultures, the rate of growth was measured daily over a period of one week. Flasks, containing equal amounts of the liquid culture medium, were inoculated with equal volumes (2% inoculum) of an actively growing forty-eight-hour old inoculum. The absorbance of the liquid cultures was determined every twenty-four hours and the dry weight was calculated from the calibration curve. It was found that maximum growth was attained at about the third day of incubation as can be noticed from Table I.

* Received August 12, 1959, from the National Research Centre, Dokki, Cairo, Egypt, U. A. R.

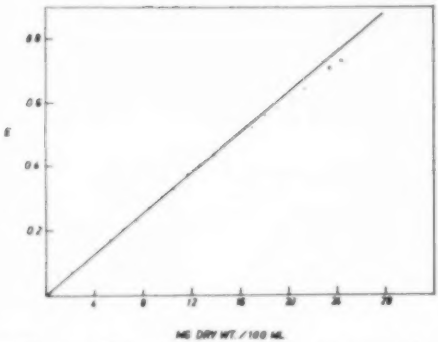


Fig. 1.—The calibration curve for converting E (1 cm., 1 μ) of a suspension of *Saccharomyces cerevisiae* into dry weight values.

TABLE I.—RATE OF GROWTH OF *S. cerevisiae* GROWN ON A LIQUID CULTURE MEDIUM

Age of Culture, days	Dry Wt., mg./100 ml.	Age of Culture, days	Dry Wt., mg./100 ml.
1	7.52	5	125.70
2	95.14	6	
3	133.70	7	130.18
4	129.70	8	126.02

It was thought that a safe period of seven days was adequate for the yeast cultures to attain full growth.

Inhibitory Effect of Preservatives When Tested Singly.—The following chemicals, namely formic (I), salicylic (II), benzoic (III), *o*-chlorobenzoic (IV), *p*-chlorobenzoic (V) acids, methyl-(VI), ethyl-(VII), propyl-(VIII), and butyl-(IX) esters of *p*-hydroxybenzoic acid, sorbic acid (X), sodium sulfite (XI), and metabisulfite (XII) were studied singly for their inhibitory effect on the growth of *Saccharomyces cerevisiae* cultures.

All the preservatives, except salicylic acid, formic acid, sodium sulfite, and metabisulfite, were prepared as alcoholic solutions and the alcohol was removed by evaporation under reduced pressure. The others were added directly to the culture medium. Preliminary experiments were done by serial dilutions in order to determine, approximately, the minimal concentration at which complete suppression of growth takes place. Thereafter, narrower dilution ranges from each of the aforementioned preservatives were prepared and tested for their inhibitory effect. When the amount of growth was plotted against the concentration of the preservative, the exact concentration at which complete inhibition takes place, could be elucidated by extrapolating the line.

Table II shows the minimal concentration from each preservative which is required to completely inhibit the growth of *Saccharomyces cerevisiae*.

From Table II, it appears that the inhibitory effect increases from formic to *p*-chlorobenzoic acid. Suppression of growth by *p*-chlorobenzoic acid was much greater than *o*-chlorobenzoic acid. Furthermore, the activity increases progressively from the methyl- to the butyl ester of *p*-hydroxybenzoic acid

TABLE II.—THE MINIMAL CONCENTRATION OF CERTAIN CHEMICALS AT WHICH COMPLETE INHIBITION OF *Saccharomyces cerevisiae* TAKES PLACE

Chemical Preservatives	Minimal Concentration Required to Inhibit Growth Completely, Gm./L.
Formic acid (I)	3.389
Salicylic acid (II)	1.266
Benzoic acid (III)	0.358
o-Chlorobenzoic acid (IV)	1.550
p-Chlorobenzoic acid (V)	0.069
Methyl ester of *p*-hydroxybenzoic acid (VI)	1.117
Ethyl ester of *p*-hydroxybenzoic acid (VII)	0.629
Propyl ester of *p*-hydroxybenzoic acid (VIII)	0.234
Butyl ester of *p*-hydroxybenzoic acid (IX)	0.101
Sorbic acid (X)	0.370
Sodium sulfite (XI)	1.250
Sodium metabisulfite (XII)	1.250

and these results are in complete agreement with those obtained by Sabalitschka in 1929 (7), Neidig and Burrell in 1944 (8), as well as by Barkley in 1959 (9). In the case of sodium sulfite or metabisulfite, erratic results were obtained and an accurate determination of the minimal inhibitory concentration was not possible. Therefore, these two chemicals were excluded from the experiments carried out with combined preservatives.

Inhibitory Effect of Combined Chemical Preservatives.—The previously mentioned chemicals were examined in combinations of two in order to study their inhibitory effects. In these experiments, half the required minimal concentration of one chemical plus varying concentrations of the other were prepared and tested for their suppressing effect on the growth of *Saccharomyces cerevisiae*. These varying concentrations ranged from 25, 50, 75, and 100% of the minimal concentration.

Out of the 10 chemicals which were tested, it was possible to prepare 90 binary combinations. The results obtained are tabulated in Table III.

From Table III it appears that salicylic acid exerts a sparing effect on the amounts required from any of the other preservatives when combined with it. As an example, the combination of formic and salicylic acids is discussed in detail: when 50% of the required concentration from salicylic acid (0.633 Gm./L.) plus varying concentrations from formic acid were used, only 0.848 Gm./L. formic (25% of the minimal concentration) was needed instead of 1.695 Gm./L. (50%) which should have been present if the effect of the combinations were only additive. Thus, a saving of at least 25% of formic acid (0.848 Gm./L.) was achieved and this combination proved to have a potentizing action. If the case was reversed, i. e., 50% of the required formic acid (1.695 Gm./L.) plus varying concentrations from salicylic acid were combined, 0.633 Gm./L. salicylic 50% was required in order to inhibit the growth completely. In the latter case the effect of the combination was only additive.

The calculation of these effects can be done in another way similar to that employed by Littlejohn and Husa (1955).

TABLE III.—THE INHIBITORY ACTION OF COMBINED BINARY MIXTURES OF CHEMICAL PRESERVATIVES[a]

Preservative With Fixed Concn. 50%	Preservative With Variable Concn., %									
	I	II	III	IV	V	VI	VII	VIII	IX	X
I	...	≤50	≤75	≤50	≤75	≤50	≤50	≤75	≤75	≤100
II	≤25	...	≤25	≤25	≤25	≤25	≤25	≤25	≤25	≤37.5
III	≤50	≤50	...	≤75	≤75	≤75	≤75	≤100	≤100	≤100
IV	≤50	≤50	≤50	...	≤50	≤75	≤75	≤100	≤100	≤75
V	≤50	≤50	≤75	≤50	...	≤75	≤75	≤75	≤75	≤75
VI	≤50	≤50	≤75	≤50	≤50	...	≤50	≤50	≤75	≤75
VII	≤50	≤50	≤100	≤75	≤75	≤50	...	≤50	≤50	≤50
VIII	≤50	≤50	≤100	≤75	≤75	≤50	≤50	...	≤50	≤75
IX	≤75	≤50	≤100	≤100	≤75	≤75	≤75	≤50	...	≤75
X	≤100	≤50	≤100	≤75	≤75	≤75	≤75	≤75	≤75	...

[a] 100% from each preservative is the lowest concentration at which complete inhibition takes place.

According to our results, 0.633 Gm. salicylic plus 0.848 Gm. formic acid should theoretically preserve 750 ml. of the culture medium. Experimentally, it was found that 0.633 Gm. salicylic plus 0.848 Gm. formic acid actually preserves 1,000 ml. culture medium. So the potentizing effect is equal to $[(1,000 \times 100)/750] - 100 = 33.3\%$. In the case of sorbic acid, the potentizing effect would be $[(1,000 \times 100)/875] - 100 = 14.3\%$.

In the same way the decrease in the effectiveness of some preservatives when combined can be calculated on the same lines. As an example, 1.695 Gm. formic plus 0.2685 Gm. of benzoic acid should preserve 1,250 ml. culture but experimentally, it was found that this mixture preserved only 1,000 ml. culture medium. Thus the decrease in the activity through combination = $[(1,000 \times 100)/1,250] - 100 = -20\%$. In other examples where the combination was even less effective than the previous one, the combination of formic (50%) and sorbic acid is discussed. Formic, 1.695 Gm. plus 0.370 Gm. sorbic acid should preserve, 1,500 ml. culture medium but, by experiment, it was found that it preserved only 1,000 ml. So the decrease in the activity through combination = $[(1,000 \times 100)/1,500] - 100 = -33.4\%$. It can be concluded that combination of salicylic (50%) with any of the following preservatives, formic, benzoic, *o*-chlorobenzoic, *p*-chlorobenzoic acid, methyl-ethyl, propyl-, and butyl esters of *p*-hydroxybenzoic acid shows a potentizing effect of at least 33.3%; with sorbic acid it was only 14.3%.

Other combinations (≤50) showed an additive or, probably, a slight potentizing effect which has not been determined. Table III also contains other combinations (≤75, ≤100) which had an inhibitory effect less than the additive one. Here the preservative action was sometimes 80% and in some cases 66.6% of the additive value. In other words, higher concentrations of these preservatives were required in order to attain complete inhibition.

SUMMARY

1. A method was devised for the spectrophotometric determination of the amount and rate of growth of *Saccharomyces cerevisiae* cultured in a semisynthetic liquid culture medium of pH 4.

2. The concentrations (Gm./L.) of formic, salicylic, benzoic, *o*-chloro- and *p*-chlorobenzoic acids, methyl-, ethyl-, propyl-, butyl esters of *p*-hydroxybenzoic acid, and sorbic acid required to inhibit the growth of the yeast was determined.

3. Combinations of half the required concentration of one preservative plus varying concentrations of the other were also tested for their suppressive action on the growth.

4. Combinations containing half the required concentration of salicylic acid plus any of the other preservatives proved to be more inhibitory than their components when tested singly.

5. Other combinations had a merely additive or even a less inhibitory action than their components.

REFERENCES

(1) Sabalitschka, T., *Mitt. Ges. Vorratsschutz*, **8,** 6(1932); cited by Littlejohn, O. M., and Husa, W. J., THIS JOURNAL, **44,** 305(1955).
(2) Schelhorn, M. V., *Deut. Lebensm. Rundschau*, **46,** 132(1950).
(3) Littlejohn, O. M., and Husa, W. J., THIS JOURNAL, **44,** 305(1955).
(4) Schimmel, J., and Husa, W. J., *ibid.*, **45,** 204(1956).
(5) Boehm, E., *Am. Perfumer Aromat.*, **73,** 37(1959).
(6) Goodwin, T. W., and Osman, H. G., *Biochem. J.*, **53,** 541(1953).
(7) Sabalitschka, T. H., *Z. angew. Chem.*, **42,** 936(1929).
(8) Neidig, C. P., Burrell, H., *Drug. & Cosmetic Ind.*, **54,** 408, 481(1944).
(9) Barkley, E. L., *Am. Perfumer Aromat.*, **73,** 33(1959).

Influence of Hydrophil-Lipophil Balance on Ointment Bases*

By JOHN W. RHYNE†, WILLIAM J. PAYNE, and CHARLES W. HARTMAN

A study of the effect of the hydrophil-lipophil balance (HLB) of ointment bases on the diffusion of water-soluble and water-insoluble drugs from the bases has been made. The diffusion of the drugs from bases having varying HLB values was determined by measuring the degree of inhibition of *Staphylococcus aureus* on serum agar plates. The drug diffusion as measured by inhibition varied considerably with change in the HLB value of the base. Phenylethyl alcohol, tyrothricin, and gramicidin gave no inhibition of *Staphylococcus aureus* in the concentrations used in the bases tested. The release of erythromycin, chlortetracycline HCl, neomycin sulfate, bacitracin, and hexachlorophene from ointment bases varied considerably with a change in the HLB value of the base.

THE HYDROPHIL-LIPOPHIL BALANCE system of classifying surface-active agents has found acceptance in pharmaceutical research relating to its applicability in the formulation of emulsions. Here it is applied to ointments to determine its effect upon drug release.

The purpose of this paper is to study the effect of the hydrophil-lipophil balance (HLB) of the base and the solubility of the medicament on drug release.

Reddish and Wales (1) conducted extensive tests on supposedly reliable antiseptic ointments of the U. S. P. X and the N. F. V and were confronted with the fact that only six of twelve U. S. P. ointments and only four of fourteen N. F. ointments showed any antiseptic activity whatever against *Staphylococcus aureus* on serum agar plates. Even phenol ointment U. S. P., containing the antiseptic upon which was based the phenol coefficient system for evaluating the efficacy of antiseptics, was found to possess negligible activity. This indicated that the type of base employed had some definite influence over release of the medicament, and subsequent research such as that of Johnston and Lee (2) has usually substantiated this belief.

Effects of Surface-Active Agents in Ointments.—The incorporation of emulsifying agents into ointment bases has been shown to have definite influence on their therapeutic performance (3, 4) and, generally speaking, this influence has been to improve the bases with respect to release of the drug and to improve the pharmaceutical elegance of the product.

The HLB System.—According to Griffin (5) the HLB value is a numerical representation of the size and strength of the hydrophilic and lipophilic groups which form the molecule of a substance. In general, the lower the HLB value of a substance, the more lipophilic is that substance; whereas with increasing HLB values there exist increasing hydrophilic properties.

The authors believe that there should be an optimum HLB at which the diffusion of the medicament from an ointment base is most efficacious since emulsifying agents may make the ointment bases more miscible with the body fluids, thereby exposing more surface area of the ointment and allowing for more effective diffusion. By a theoretical extension of the HLB system, it should be possible to approximate the HLB of all the components of a base, including the therapeutically-active ingredients themselves, according to their hydrophilic and lipophilic tendencies and their solubility in water or organic solvents. Then one would be able to formulate an ointment that has an HLB value nearer the ideal with regard to release of the drug. A variation of the HLB within an ointment should give a corresponding variation in the release of medicament from that ointment. Upon inunction, it is assumed that at least part of the ointment is emulsified with tissue fluids. For different HLB values the type of emulsion and the particle size should be different.

To effect a practical application of this theory of HLB influence on drug release from ointments, some simple procedure for determining the HLB value of the ingredients (and the effect of the value of the HLB of the ointment) is needed. Some work in this area has been done by Chun, *et al.* (6), to determine the approximate HLB

* Received from the University of Georgia, School of Pharmacy, Athens.

Abstracted in part from a thesis presented to the Graduate School, University of Georgia, in partial fulfillment of the requirements for the degree of Master of Science.

Adapted from the manuscript submitted by John W. Rhyne which received honorable mention in the 1958 Lunsford Richardson Pharmacy Awards competition.

† Present Address: Alcon Laboratories, Fort Worth, Texas.

Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.

of various natural emulsifying agents when in the presence of surface-active agents of known HLB values. Their method can be adapted to determine the HLB of other substances as well.

By using the equation of Chun, *et al.* (6), in combination with the method of Griffin (5), the HLB value of an ointment formula, including all the constituents, might be determined and if the most effective HLB value for release of the particular drug were known, the HLB could then be adjusted by addition of suitable surface-active agents of known HLB to give an ointment base with an HLB value suitable for optimum drug release.

It is believed that drugs with different distribution coefficients in ointments with different HLB values would show a variation in drug release from the bases.

An attempt will be made to ascertain how a variation in HLB of the base will affect the diffusion of a drug from the base. In the future this may serve as a guide for the selection of a base with a definite HLB value which will give optimum drug release from an ointment.

TABLE I.—OINTMENT BASES, COMPOSITION, CONSISTENCY AT 26° C., AND HLB VALUE

Surface-Active Agents Used[a, b]	Lot No.	HLB[c]	Consistency of Penetration, mm.[d]
Sorbitan trioleate	5241A	1.8	271.4
Sorbitan sesquioleate	6404C	3.7	270.1
Sorbitan monostearate	6539C	4.7	241.0
Sorbitan monopalmitate	32F	6.7	265.6
Sorbitan monolaurate	109	8.6	285.4
Polyoxyethylene sorbitan monostearate	185	9.6	279.6
Polyoxyethylene sorbitan monooleate	355	10.0	271.0
Polyoxyethylene sorbitan trioleate	297	11.0	285.4
Polyoxyethylene sorbitan monolaurate	6508B	13.3	272.7
Polyoxyethylene sorbitan monooleate	451	15.0	280.3
Polyoxyethylene sorbitan monopalmitate	185	15.6	287.6
Polyoxyethylene lauryl ether	219	16.7	278.0
Polyoxyethylene stearate	5725B	17.9	253.7

[a] All ointment bases consisted of 90% petrolatum U. S. P. and 10% surface-active agents.
[b] Products furnished by the Atlas Powder Co., Wilmington, Del.
[c] The HLB value is that assigned by Atlas Powder Co.
[d] All consistencies represent the average value obtained by the U. S. P. XV method.

EXPERIMENTAL

Determination of Drug Release.—A modification of the agar plate method of the Food and Drug Administration (7) as described by Plaxco and Husa (8) was employed.

Preparation of Ointments.—The primary base used in all ointments was white petrolatum U. S. P. Using this base, a series of HLB bases was prepared using emulsifying agents with HLB values varying from 1.8 to 17.9. Ten per cent, by weight, of surface-active agents of the polyoxyethylene, polyoxyethylene sorbitan, sorbitan series, having established HLB values as shown in Table I, were incorporated. The consistency of these bases shown in Table I, was checked by the U. S. P. XV (9) method using a Precision Penetrometer (serial number 1–4, manufactured by Precision Scientific Co., Chicago, Ill.). It was desired to have the consistencies of the bases fall within or very near the range of consistency allowed for petrolatum, U. S. P.

Inhibition studies were carried out to determine if the variation in consistency of the base would cause a difference in the degree of inhibition. No significant difference in inhibition in the range of consistencies used was observed.

All of the drugs were incorporated into the bases by levigation. No levigating or solubilizing agents were used.

Drugs having different solubilities in water were chosen. Thus erythromycin, hexachlorophene, tyrothricin, and gramicidin are insoluble, whereas chlortetracycline HCl, neomycin sulfate, and phenylethyl alcohol are soluble in water. The drugs were incorporated into the bases on a weight in weight per cent basis as follows: erythromycin 1.0, chlortetracycline HCl 3.0, neomycin sulfate 0.6, hexachlorophene 0.45, tyrothricin 0.1, gramicidin 0.05, and phenylethyl alcohol 5.0.

Controls.—Controls consisted of bases of identical HLB values with no medicament, petrolatum alone, and petrolatum with medicament. All controls were treated in the same manner as the HLB ointments containing medicaments.

All ointments were irradiated by ultraviolet light during the manufacturing process and then placed in ointment tubes which had also been irradiated to effect a state of near-sterility.

RESULTS

General.—Phenylethyl alcohol, tyrothricin, and gramicidin used in therapeutic concentrations equivalent to those commonly used in commercial ointments gave no significant inhibition of *Staphylococcus aureus* at the HLB values tested in these experiments.

Neomycin Sulfate.—As seen in Fig. 1, neomycin sulfate in all three trials gave better inhibition in the ointments with HLB values between 8 and 16. Therefore, neomycin sulfate must have diffused more readily between the HLB value of 8 and 16

Chlortetracycline Hydrochloride.—As shown in Fig. 1, chlortetracycline hydrochloride gave greater inhibition in ointments with high HLB values.

Erythromycin.—As seen in Fig. 2, the erythromycin ointments with low HLB values gave greater inhibition.

Hexachlorophene.—Figure 2 shows that for hexachlorophene the inhibition is almost constant except for two apparent peaks at HLB 8.6 and HLB 10.0, with the least inhibition found at HLB 9.6 and 17.9.

All drugs showed a decrease in inhibition at HLB 17.9.

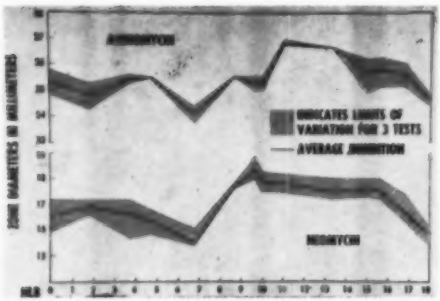


Fig. 1.—Inhibition of *Staphylococcus aureus* by Aureomycin hydrochloride and neomycin sulfate ointments with varying HLB values.

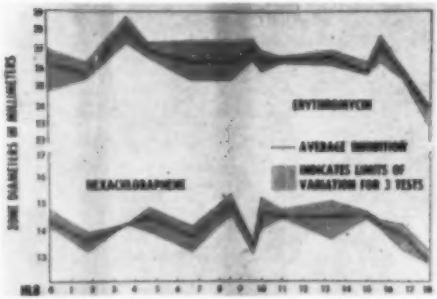


Fig. 2.—Inhibition of *Staphylococcus aureus* by erythromycin and hexachlorophene ointments with varying HLB values.

Controls.—No inhibition was observed for the control ointments containing petrolatum, U. S. P. and petrolatum with the various surface-active agents.

It was thought that the differences in inhibition as shown in these experiments might be due to experimental error, but it may be seen that in all three determinations for each drug the variations in inhibition are similar. Average curves were drawn using the three test results but variation did not differ significantly from the individual curves as shown by the upper and lower limits. It was also considered possible that the variation in inhibition might be due to variation in consistency, but experiments with varying consistencies did not serve to substantiate this. It is felt that factors other than HLB should be considered in further work. The distribution coefficient of the drug and the possibility of intermolecular activity may well play an important part in the diffusion of drugs from ointment bases.

CONCLUSIONS

The HLB of an ointment base has some influence on the diffusion of the medicament from that base. From this work it may be surmised that the HLB value of an ointment base should be considered when attempting to adjust that base to increase or decrease the rate of drug release. Insufficient data does not warrant a conclusion concerning the effect of the solubility of the drug on release from bases having different HLB values.

REFERENCES

(1) Reddish, G. F., and Wales, H. J., THIS JOURNAL, **18**, 576(1929).
(2) Johnston, G. W., and Lee, C. O., *ibid.*, **32**, 278(1943).
(3) McDonald, L. H., and Himelick, R. E., *ibid.*, **37**, 368(1948).
(4) Dodd, M. C., Hartmann, F. W., and Ward, W. C., *ibid.*, **35**, 33(1946).
(5) Griffin, W. C., *J. Soc. Cosmetic Chemists*, **1**, 311(1949).
(6) Chun, A. H. C., Joslin, R. S., and Martin, A. N., *Drug & Cosmetic Ind.*, **82**, 164(1958).
(7) "The Compilation of Regulations for Tests and Methods of Assay for Antibiotic Drugs," U. S. Dept. of Health, Education, and Welfare, Food and Drug Administration, April 1951. Sec. 141.401 and 141.402.
(8) Plaxco, J. M., Jr., and Husa, W. J., THIS JOURNAL, **45**, 141(1956).
(9) "U. S. Pharmacopeia," 15th Rev., Mack Publishing Co., Easton, Pa., 1955, p. 930.

The Interaction of Citrate With Aspirin and Benzoic Acid*

By SANFORD BOLTON

Solubility studies were used to determine the nature and extent of the interaction between sodium citrate and aspirin. Because of the complex nature of this system, parallel studies of the solubilizing action of sodium citrate and acetate on benzoic acid were made for purposes of comparison. The acetate-benzoic acid reaction closely followed results expected from mass law considerations over a wide concentration range. However, the weak acid-citrate interactions could be described by the mass law only at low citrate concentrations, showing wide deviations at higher concentrations. It was apparent that the deviations were a function of many variables which included changes of solubility and Ka with the nature and concentration of the solution species. The fact that the interactions could not be rationalized by these changes alone at very high citrate concentrations suggested an association or associations among the dissolved species.

IT HAS BEEN long recognized that aspirin can be solubilized in aqueous solutions of citrate salts. Such combinations have been shown to produce more stable solutions of aspirin than those obtained when solubilization is effected by other bases (1). Although the increase of aspirin stability in these systems has been suggested for some time, no specific investigation which could explain this phenomenon has been reported. It was the purpose of the present study to attempt such an investigation.

The experimental approach consisted of noting changes in the solubility and pH of saturated solutions of aspirin in the presence of varying amounts of citrate and strong base. The observed results were to be compared with results expected theoretically. Possible association of species in this system, which could explain the increased stability, would be indicated by profound deviations from expected results. Because this type of interaction is very complex, parallel studies were made with benzoic acid.[1] This acid was chosen because of its structural similarity to aspirin and because of the large quantity of data available on the effect of environment on its activity in aqueous solutions. Certain concurrent studies were made in order to help elucidate the above reactions. These included (*a*) the effect of citric acid on the solubilities of undissociated aspirin and benzoic acid, (*b*) salt effects, and (*c*) the effect of concentration on the Ka values of citric acid.

* Received August 21, 1959, from the College of Pharmacy, University of Rhode Island, Kingston.
Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.

[1] Doosag and Bhagwat (2) had followed the interaction of benzoic acid with citrate and acetate at low concentrations in order to determine dissociation constants. In the present study, more complete data are presented at both low and high salt concentrations.

THEORY

A paper by Higuchi, Gupta, and Busse (3) has described equations for the calculation of the solubility of difficultly soluble, weakly acidic drugs as a function of pH. Equation 1 was found to be approximately valid for a variety of weak acids in solutions of constant ionic strength when S and S_o were expressed as concentrations rather than activities.

$$\log (S - S_o) = \log S_o - \mathrm{pKa} + \mathrm{pH} \quad \text{(Eq. 1)}$$

S = Total solubility and S_o = solubility of undissociated acid.

The interaction of a weak acid and weak base may be represented by the following equations:

$$\mathrm{HA} + \mathrm{B}^- \leftrightarrows \mathrm{HB} + \mathrm{A}^- \quad \text{(Eq. 2)}$$

$$\mathrm{K} = \mathrm{Ka/Kb} = (\mathrm{HB})(\mathrm{A}^-)/(\mathrm{HA})(\mathrm{B}^-) \quad \text{(Eq. 3)}$$

where

$$\mathrm{Ka} = (\mathrm{A}^-)(\mathrm{H}^+)/(\mathrm{HA}) \quad \text{(Eq. 4)}$$

and

$$\mathrm{Kb} = (\mathrm{B}^-)(\mathrm{H}^+)/(\mathrm{HB}) \quad \text{(Eq. 5)}$$

If HA represents a slightly soluble acid and if the system is saturated with this acid, Eq. 3 may be expressed as

$$\mathrm{Ka}(S_o)/\mathrm{Kb} = (\mathrm{HB})(\mathrm{A}^-)/(\mathrm{B}^-) \quad \text{(Eq. 6)}$$

As is the case in Eq. 1, this relationship is valid only when concentrations are replaced by activities. In dilute solutions, activity generally approximates concentration; but in concentrated solutions, deviations from Eqs. 1 and 6 are to be expected. The magnitude and direction of such deviations are dependent, generally, on the nature of the solution species.

EXPERIMENTAL

Reagents.—Recrystallized aspirin, m. p. 133°; benzoic acid, m. p. 122°; sodium acetate, anhydrous; sodium chloride, anhydrous; sodium citrate, dihydrate U. S. P.; citric acid, monohydrate U. S. P.; sodium hydroxide solution, carbonate free.

Procedure.—An excess of aspirin or benzoic acid was shaken with 10 ml. of solution containing varying amounts of the appropriate reagents in a constant temperature water bath at 30°. The aspirin solutions were shaken for three hours to keep hydrolysis to a minimum. The benzoic acid solutions were shaken for twelve hours. The pH of each solution, then, was determined by use of a Beckman model G pH meter, and clear aliquots were diluted with 0.02 *N* hydrochloric acid for spectrophotometric analysis. In the salting out studies and in the studies concerned with the effect of citric acid on the solubilities of benzoic acid and aspirin, 0.02 *N* hydrochloric acid was added to keep dissociation of the acids to a minimum.

Analysis. The peak at 273 mμ in the U. V. spectra was used as a basis for the analysis of benzoic acid. In the aspirin studies, the amount of salicylic acid formed was determined by its peak at 302.5 mμ. The minimum in the U. V. spectrum at 259 mμ was then used to determine the amount of aspirin in solution.

RESULTS AND DISCUSSION

Citric Acid-Aspirin and Citric Acid-Benzoic Acid Interactions.—The effect of citric acid on the solubilities of undissociated aspirin and benzoic acid is shown in Fig. 1. The apparent interaction between these uncharged species is slight. The results, however, suggest the possibility of associations in solutions of higher pH where charged basic moieties may exist. Kolthoff and Bosch (4) and Larsson (5) have noted the presence of this type of complex in the benzoic acid-benzoate system.

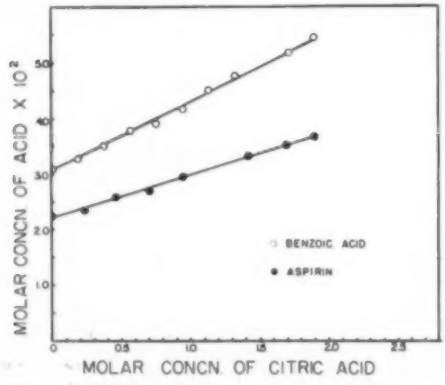


Fig. 1.—The effect of citric acid on the solubilities of undissociated benzoic acid and aspirin at 30°.

Salt Effects.—The extent of interaction in the weak base-insoluble weak acid reactions depends on the values of Ka, Kb, and S_o (Eq. 6). These values are greatly dependent on the nature of the ionic environment. It is virtually impossible to determine these effects in the systems investigated in this report. It appears that the best approach in an analysis of these systems would be to use the results of salt effects in relatively simple systems as an approximation of such effects in the present investigation.

The influence of sodium chloride on the solubilities of the weak acids is depicted in Table I and Fig. 2. In the case of benzoic acid, these results agree closely with those obtained by Kolthoff and Bosch (4) at 25°.

TABLE I.—THE EFFECT OF SODIUM CHLORIDE ON THE SOLUBILITY OF BENZOIC ACID AND ASPIRIN AT 30°

Ionic Strength	Benzoic Acid Solubility, Moles/L.	Ionic Strength	Aspirin Solubility, Moles/L.
0	0.031	0	0.022
0.276	0.0271	0.171	0.0216
0.550	0.0239	0.430	0.0190
0.824	0.0211	0.514	0.0169
1.12	0.0189	0.600	0.0174
1.39	0.0171	0.684	0.0165
1.66	0.0159	0.770	0.0161
1.93	0.0141	0.855	0.0148
2.21	0.0125	1.03	0.0139
2.49	0.0112	1.20	0.0130
2.76	0.0102	1.37	0.0116
		1.54	0.0106
		1.71	0.0102
		1.90	0.0086
		2.06	0.0088
		2.40	0.0072
		2.74	0.0068
		3.08	0.0051
		3.42	0.0045

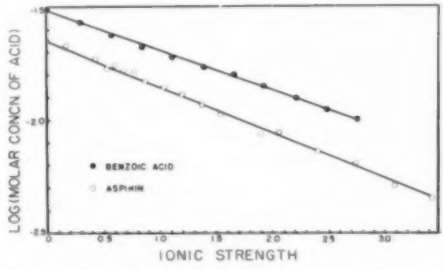


Fig. 2.—The effect of sodium chloride on the solubilities of undissociated benzoic acid and aspirin at 30°.

Although no studies of salt effects on Ka values were made in this study, a review of some pertinent data is included here. Riesch and Kilpatrick (6) and Guntelberg and Schrodt (7) have shown that the apparent Ka of benzoic acid increased with salt concentration to a maximum value of 11.9×10^{-6} at 0.5 *M* sodium chloride. At higher ionic strengths, the Ka gradually decreased to 2.46×10^{-6} at 5.0 *M* sodium chloride.

Several investigators have published data on the effect of foreign neutral salts (8, 9), as well as the effect of acetate (10) on the Ka of acetic acid. Again, a maximum in Ka was found to occur at approximately 0.5 *M* sodium chloride. In the presence of sodium acetate, similar results were obtained, the maximum being dependent both on the amount of acetate present and on the ratio of acetate to acetic acid. The original publications should be consulted for more detail.

The Effect of Concentration on the Dissociation Constants of Citric Acid.—Adell (11) has demonstrated that the apparent values of the Ka's for citric acid increase in the presence of sodium chloride. Kolthoff and Bosch (12) showed that the Ka's also increase with the concentration of citric acid and its corresponding potassium salts. Since their studies involved total citric acid concentrations which were comparatively small, an extension of these studies, with sodium citrate, to higher concentrations was made. The results are shown in Table II. The present work was not intended to yield exact results. However, the values do give a good approximation of variation of the apparent values of the Ka's with respect to concentration. The Ka's were determined by potentiometric titrations, utilizing the one-half neutralization point in the titration curves.[2]

TABLE II.—CONCENTRATION EFFECTS ON THE DISSOCIATION CONSTANTS OF CITRIC ACID

Molar Concn. of Citric Acid and Dihydrogen Citrate[a]	Apparent pK_{a_1}
0.0115	3.03
0.091	2.97
0.232	2.86
0.455	2.79
0.910	2.55
Molar Concn. of Dihydrogen Citrate and Monohydrogen Citrate	**Apparent pK_{a_2}**
0.011	4.55
0.083	4.34
0.221	4.19
0.415	4.12
0.835	3.91
Molar Concn. of Monohydrogen Citrate and Citrate	**Apparent pK_{a_3}**
0.011	5.89
0.077	5.62
0.212	5.36
0.334	5.26
0.765	5.13

[a] A concentration of 0.0115 means that each species is present in approximately this concentration.

Weak Acid-Base Interactions.—The solubility of aspirin and benzoic acid as a function of pH in the presence of various bases is shown in Figs. 3 and 4 and Tables III and IV. (See also Tables V, VI, and VII.) The solid lines in Figs. 3 and 4 represent values calculated from Eq. 1.

The results of the solubilization of the acids by sodium hydroxide conform closely to the calculated values, showing some deviation at high concentrations.

The acetate-benzoic acid interaction also followed closely results expected from Eq. 1. It may be noted that the position of the straight lines of slope 1 in Figs. 3 and 4 is determined by the "y" intercept, log *So* - pKa. Increased ionic environment, in the acetate-benzoic acid system results in a simultaneous decrease in the values of log *So* and pKa for benzoic acid. This compensatory effect would tend to fix the position of the straight line.

[2] Appropriate corrections for the effect of other ionization constants were made according to a method suggested by Kolthoff and Bosch (12).

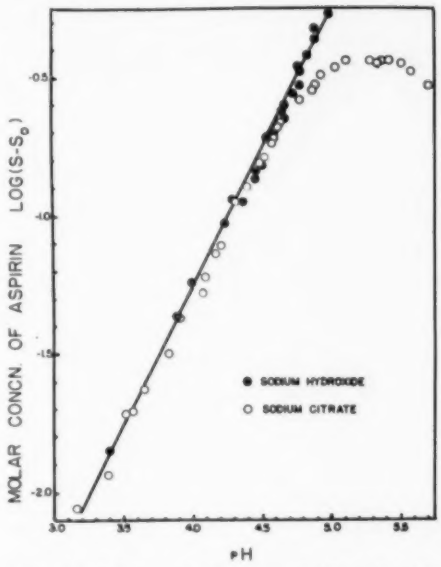


Fig. 3.—Influence of pH on the solubility of aspirin in the presence of sodium hydroxide and sodium citrate at 30°. The solid line represents values calculated from Eq. 1.

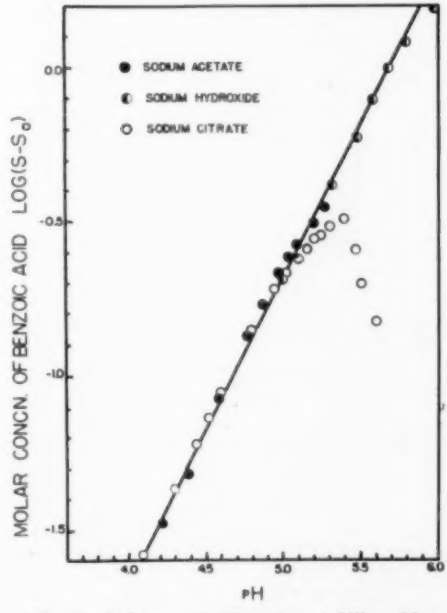


Fig. 4.—Influence of pH on the solubility of benzoic acid in the presence of sodium hydroxide, sodium citrate, and sodium acetate at 30°. The solid line represents values calculated from Eq. 1.

TABLE III.—SOLUBILITY OF ASPIRIN AS A FUNCTION OF pH AT 30°

Final pH	Solubility, Moles/L.
1.76[a]	0.022
2.66[a]	0.024
3.40	0.036
3.90	0.076
4.00	0.079
4.20[a]	0.098
4.24	0.116
4.30	0.138
4.38	0.135
4.47	0.168
4.47	0.156
4.56[a]	0.180
4.52	0.175
4.55	0.212
4.60	0.222
4.66	0.261
4.68	0.275
4.68	0.244
4.74	0.295
4.78	0.372
4.79	0.316
4.80	0.352
4.84	0.403
4.90	0.458
4.90	0.504
5.00	0.554
5.10[a]	0.610
5.10[a]	0.610

[a] These points were omitted from Fig. 3 due to spatial limitations.

TABLE IV.—SOLUBILITY OF BENZOIC ACID AS A FUNCTION OF pH AT 30°

Final pH	Solubility, Moles/L.
2.85[a]	0.032
5.01	0.239
5.33	0.445
5.50	0.630
5.60	0.817
5.70	1.027
5.82	1.249
6.00	1.600
6.23[a]	2.110
6.36[a]	2.470

[a] These points were omitted from Fig. 4 due to spatial limitations.

Thus, the apparent agreement with Eq. 1 in this case may be merely coincidental.

In the cases of the citrate-weak acid systems, Eq. 1 describes the interactions only at low citrate concentrations. The decrease in solubility at high citrate concentrations was not due to an insoluble complex as was shown by analysis of the solid phase in appropriate containers.

Figures 5, 6, and 7 and Tables V, VI, and VII show the effect of citrate and acetate concentration on the solubilities of aspirin and benzoic acid. The observed results are compared with values calculated from Eq. 6.

With the assumption that the ionic species in solution exert effects similar to sodium chloride, the value of $Ka(So)/Kb$ (Eq. 6) can be approximated and the extent of interaction calculated. It is of interest to note that in the acetate-benzoic acid system such calculations[3] yield a nearly constant value for $Ka(So)/Kb$, which might explain the near coincidence of the curves in Fig. 7.

TABLE V.—SOLUBILITY OF ASPIRIN AS A FUNCTION OF SODIUM CITRATE CONCENTRATION AT 30°

Sodium Citrate, Moles/L.	Aspirin Solubility, Moles/L.	pH
0.0034	0.0306	3.16
0.0068	0.0332	3.39
0.0085	0.0407	3.51
0.0102	0.0412	3.57
0.0136	0.0453	3.65
0.0170	0.0533	3.83
0.0255	0.0646	3.91
0.0340	0.0746	4.08
0.0425	0.0830	4.10
0.0510	0.0954	4.18
0.0595	0.0993	4.21
0.0850	0.135	4.32
0.102	0.149	4.40
0.136	0.178	4.50
0.153	0.183	4.53
0.170	0.203	4.58
0.204	0.213	4.60
0.255	0.243	4.65
0.332	0.284	4.79
0.408	0.306	4.88
0.510	0.317	4.90
0.545	0.343	4.95
0.680	0.366	5.05
0.816	0.386	5.13
1.090	0.382	5.30
1.190	0.379	5.36
1.224	0.386	5.38
1.360	0.389	5.44
1.530	0.378	5.53
1.700	0.354	5.60
1.900	0.319	5.72

TABLE VI.—SOLUBILITY OF BENZOIC ACID AS A FUNCTION OF SODIUM CITRATE CONCENTRATION AT 30°

Sodium Citrate, Moles/L.	Benzoic Acid Solubility, Moles/L.	pH
0.0136	0.0572	4.09
0.0272	0.0747	4.30
0.0408	0.0917	4.44
0.0544	0.105	4.52
0.068	0.120	4.60
0.136	0.171	4.80
0.204	0.223	4.94
0.272	0.246	5.04
0.340	0.270	5.11
0.408	0.288	5.17
0.476	0.309	5.21
0.544	0.316	5.26
0.680	0.332	5.33
0.850	0.351	5.41
1.190	0.288	5.49
1.360	0.231	5.52
1.700	0.180	5.61

Similar considerations may be used to approximate the extent of interaction in the citrate-benzoic acid reaction. The value, $Ka(So)/Kb$, would be expected to decrease at high ionic strengths, since Ka and So for benzoic acid would decrease and Kb,

[3] The data used for these calculations were taken from references (9) and (11) as well as from the present study.

TABLE VII.—SOLUBILITY OF BENZOIC ACID AS A FUNCTION OF SODIUM ACETATE CONCENTRATION AT 30°

Sodium Acetate, Moles/L.	Benzoic Acid Solubility, Moles/L.	pH
0.0428	0.0645	4.22
0.0713	0.0792	4.39
0.143	0.115	4.59
0.285	0.166	4.78
0.429	0.203	4.88
0.570	0.246	4.98
0.713	0.275	5.05
0.858	0.295	5.10
1.140	0.344	5.21
1.430	0.383	5.28

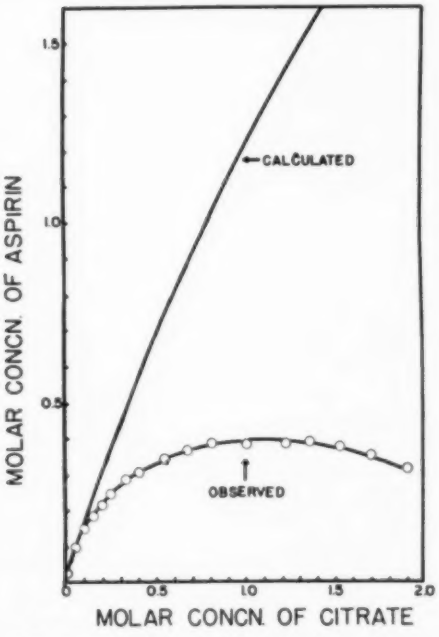


Fig. 5.—The effect of sodium citrate on the solubility of aspirin at 30°. Observed values are compared with those calculated from Eq. 6.

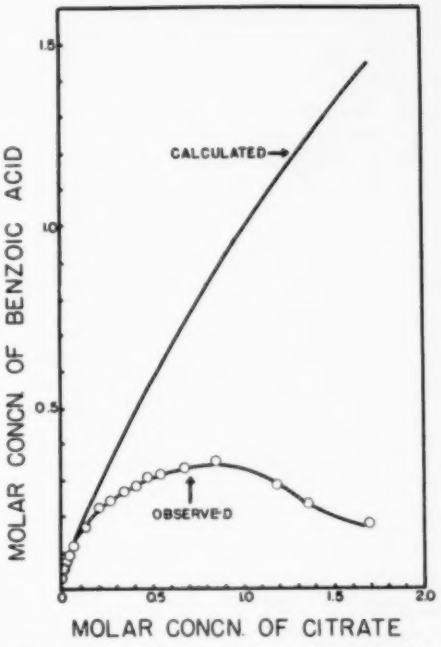


Fig. 6.—The effect of sodium citrate on the solubility of benzoic acid at 30°. Observed values are compared with those calculated from Eq. 6.

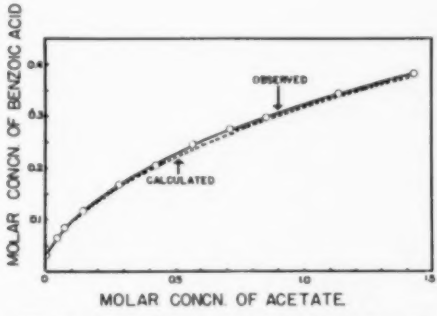


Fig. 7.—The effect of sodium acetate on the solubility of benzoic acid at 30°. Observed values are compared with those calculated from Eq. 6.

the third dissociation constant for citric acid,[4] would increase. The apparent decrease in the interaction at high citrate concentrations as observed in Figs. 5 and 6 may be explained on this basis.

A further analysis of the experimental results in solutions of high citrate concentration can yield an approximate value for the third dissociation constant of citric acid. In the absence of interfering reactions, the amount of benzoate formed would be equal to the amount of monohydrogen citrate in solution. This fact, the knowledge of the amount of citrate added to the system, and the observed pH can be used to calculate the constant. The values so obtained revealed abnormally high dissociation constants at high citrate concentrations in the benzoic acid interaction. For example, at 1.70 *M* citrate, the calculated value of Ka_3 was approximately 2×10^{-4}. If citrate ions were being used up in competing reactions, such as association with itself or other solvent species, the above observations could be readily accounted for.

It appears that similar considerations would apply to the citrate-aspirin system due to the similarity of the acids and the experimental results. Since a citrate complex cannot be actually demonstrated, especially at low concentrations, it is difficult to rationalize previous reports of increased stability of aspirin in citrate solutions.

[4] At high concentrations of citrate, the predominant reaction is benzoic acid + citrate$^{=}$ $\rightleftharpoons$ benzoate^{-} + H citrate^{-}.

SUMMARY

1. The solubility of benzoic acid and aspirin as a function of pH, citrate concentration, and sodium chloride concentration has been determined. In addition, the effect of sodium acetate on the solubility of benzoic acid and the effect of concentration on the dissociation constants of citric acid were investigated.

2. The interaction of sodium acetate and benzoic acid closely followed the law of mass action. The citrate interactions deviated widely from such considerations; an actual decrease in solubility was observed at high citrate concentrations. These results, however, are to be expected if the effect of ionic environment is taken into consideration.

3. Analysis of the acid-base interactions with the aid of other studies, pursued here and elsewhere, suggests that complexing occurs to only a limited extent, if at all, except at very high citrate concentrations where such associations may play a predominant role in the interactions.

REFERENCES

(1) Bowey, A. E., *Pharm. J., New Zealand*, **19,** 11(1957).
(2) Doosag, S. S., and Bhagwat, W. V., *J. Indian Chem. Soc.*, **10,** 225(1933).
(3) Higuchi, T., Gupta, M., and Busse, L. W., THIS JOURNAL, **42,** 157(1953).
(4) Kolthoff, I. M., and Bosch, W., *J. Phys. Chem.*, **36,** 1685 (1932).
(5) Larsson, E., *Z. physik. Chem.*, Abt. A, **153,** 456(1931).
(6) Riesch, L., and Kilpatrick, M., *J. Phys. Chem.*, **39,** 891(1935).
(7) Guntelberg and Schrodt, *Z. physik. Chem.*, **135,** 393 (1928).
(8) Eillila, A., *Acta Chem. Scand.*, **6,** 1562(1952).
(9) Kiss, A. V., and Urmancy, A., *Z. physik. Chem.*, Abt. A, **171,** 257(1935).
(10) Cohn, E. J., Heyroth, F. F., and Meakin, M. F., *J. Am. Chem. Soc.*, **50,** 696(1928).
(11) Adell, B., *Z. physik. Chem.*, Abt. A, **187,** 65(1940).
(12) Kolthoff, I. M., and Bosch, W., *Rec. trav. chim.*, **47,** 558(1928).

Veratrum Alkaloids XLIII*

The Structure of Cevadine

By S. MORRIS KUPCHAN and ADRIANO AFONSO

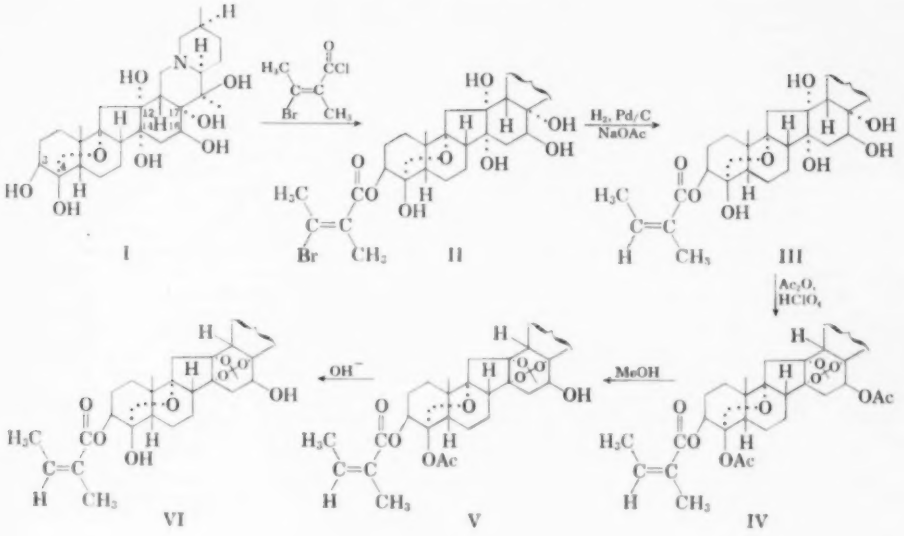
The structure of cevadine, the principal alkaloid of *Schoenocaulon officinale* A. Gray, has been elucidated as veracevine 3-angelate (III). Acylation of veracevine with a limited amount of 3-bromoangeloyl chloride afforded veracevine 3-(3′-bromoangelate) (II). The latter compound, on hydrogenolysis, afforded veracevine 3-angelate which was found to be identical with cevadine.

CEVADINE is the most abundant alkaloid of commercial veratrine, an insecticidal mixture of alkaloids obtained from the seeds of *Schoenocaulon officinale* A. Gray (sabadilla seed). It was first isolated by G. Merck (1) in 1855 and further characterized by Schmidt and Koppen (2) and by Wright and Luff (3). Wright and Luff hydrolyzed cevadine with dilute alcoholic alkali and isolated an amorphous alkamine and tiglic acid. Bosetti (4) and Ahrens (5) each reported the alkaline hydrolysis of cevadine to yield an amorphous alkamine and the geometric isomer of tiglic acid, *viz.* angelic acid. Freund and Schwarz (6) hydrolyzed cevadine with hot concentrated alcoholic potassium hydroxide, obtained the crystalline alkamine cevine and a mixture of tiglic and angelic acids, and concluded that cevadine is an angelate ester of cevine. Horst (7) disputed the latter conclusion on the basis of his finding that hydrolysis under acidic conditions gave only tiglic acid. Stoll and Seebeck (8) hydrolyzed cevadine under somewhat milder alkaline conditions than those employed previously, obtained the crystalline alkamine cevagenine and angelic acid, and concluded that cevadine is an angelate ester of cevagenine. Subsequent work by Pelletier and Jacobs (9) and by our group (10) showed that saponification of cevadine under still milder conditions afforded the alkamine veracevine (I), and supported the view that the alkamine present in cevadine is veracevine. While circumstantial evidence has made possible a tentative formulation for ceva-

* Received November 9, 1959, from the Department of Pharmaceutical Chemistry, University of Wisconsin, Madison.

Supported in part by grants from the National Institutes of Health [H-2275 (C4)] and the Wisconsin Alumni Research Foundation.

Part XLII in the series: S. M. Kupchan, C. I. Ayres, and R. H. Hensler, *J. Am. Chem. Soc.*, in press.



dine (11), the nature of the alkamine, the acid residue, and the point of attachment of the acid residue to the alkamine nucleus have not been settled unequivocally. In the present report evidence is presented for definite assignment of the veracevine 3-angelate structure (III) to cevadine.

That veracevine and angelic acid are indeed the true components of the natural ester alkaloid was established by the synthesis from veracevine of an angelate ester identical with cevadine. Veracevine (I) was treated with a limited amount of 3-bromoangeloyl chloride (12, 13, 14) to yield veracevine 3-(3′-bromoangelate) (II), $[\alpha]_D^{21} -4°$ (ethanol). The latter compound, on hydrogenolysis, afforded veracevine 3-angelate (III), m. p. 208–209°, $[\alpha]_D^{24} +13°$ (ethanol). This product showed no depression in melting point on admixture with an authentic sample of cevadine. The infrared spectra and paper chromatographic behavior of the respective samples were identical.

Cevadine has been regarded as a 3-acyl derivative of veracevine [see, e. g., (11)] on the basis of our observation in October 1953 (heretofore unpublished) that periodic acid cleavage affords an amorphous product which shows no γ-lactone absorption in the infrared. Our result indicated that the acyl residue blocked the C_3, C_4 α-ketol hemiketal system toward periodic acid attack. However, the latter experiment did not distinguish between a C_3 and a C_4 ester structure for cevadine. We deemed the presence of an acyl moiety at C_4 as highly unlikely on biogenetic grounds, for no C_4 ester has been found among the multitude of naturally occurring ester derivatives of the veratrum alkamines zygadenine, germine, or protoverine (13–19). Nevertheless, it was considered desirable to seek experimental evidence in favor of the C_3 ester structure (III) by chromic acid titration experiments. Cevadine D-orthoacetate (VI) (20) demonstrated a chromic acid consumption which paralleled that of cevadine D-orthoacetate monoacetate (V) and indicated the presence of one secondary hydroxyl group. C_4 was thereby excluded as a site for attachment of the angelate ester of cevadine, for a C_4-attachment would leave two chromic acid-sensitive secondary hydroxyl groups in cevadine D-orthoacetate. The combination of the results reported herein strongly support the veracevine 3-angelate structure (III) for cevadine.

EXPERIMENTAL

Melting points are corrected for steam exposure. Infrared spectra were determined on a Baird model B double beam infrared recording spectrophotometer and chloroform was used as the solvent. Paper chromatograms were run by the descending technique employing Whatman No. 1 paper.

Veracevine 3-(3′-Bromoangelate) (II).—Veracevine (I, 1.02 Gm., m. p. 220–225° (decompn.) after softening at 179–183°) was dissolved in pyridine (5 cc.) and the stirred solution was treated at room temperature with 3-bromoangeloyl chloride (0.60 Gm.) (12). The mixture was stirred for sixteen hours, cooled with crushed ice, brought to pH 8 with 10% sodium carbonate solution, and extracted thoroughly

with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was dissolved in benzene and the solution was evaporated to dryness under reduced pressure; the procedure was repeated until the residue no longer smelled of pyridine. The brownish-yellow residue was triturated with ether (25 cc.) and the insoluble solid (0.36 Gm.,) identified as veracevine, removed by filtration. The filtrate was evaporated to dryness and the pale yellow residue (0.74 Gm.) chromatographed on Merck acid-washed alumina (14 Gm.). The progress of the column separation was followed by paper chromatography employing the procedure of Levine and Fischbach (21) [solvent system: *n*-butyl acetate, *n*-butanol, formic acid (25:5:1 by volume)]. Elution with 50% benzene-chloroform (200 cc.) yielded an amorphous residue (0.10 Gm.) which appeared to consist of a mixture of mono and diesters. Elution with chloroform (500 cc.) yielded veracevine (3-3′-bromoangelate) (0.26 Gm.) as a pale yellow amorphous solid, $[\alpha]_D^{23}$ −4° (c 1.36 ethanol). Further elution with 2% methanol-chloroform yielded veracevine.

Hydrogenolysis of Veracevine 3-(3′-Bromoangelate): Synthesis of Cevadine (III).—To a suspension of 10% palladium on carbon (0.07 Gm.) in 95% ethanol (10 cc.) which had previously been saturated with hydrogen, was added a solution of veracevine 3-(3′-bromoangelate) (II, 0.14 Gm.) in ethanol (4 cc.) containing anhydrous sodium acetate (0.07 Gm.). Hydrogenation was allowed to proceed at room temperature and atmospheric pressure until one mole equivalent of hydrogen had been consumed (*ca.* three minutes). The catalyst was removed by filtration, washed with ethanol (10 cc.), and the filtrate evaporated to dryness under reduced pressure. The residue was suspended in ice water (2 cc.), made alkaline with 10% sodium carbonate solution, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield a resin (0.12 Gm.) which was crystallized from acetone-water to yield cevadine (85 mg.), m. p. 208–209°, $[\alpha]_D^{23}$ +13° (c 0.92, ethanol). The melting point was not depressed on admixture with an authentic sample of cevadine. The infrared spectra and paper chromatographic behavior of the respective samples were identical.

Periodic Acid Oxidation of Cevadine.—Cevadine (III, 2 Gm.), m. p. 208–210°, dissolved in methanol (25 cc.) and water (25 cc.), was treated with periodic acid (1 Gm.). After twenty hours at room temperature, the solution was concentrated under reduced pressure to remove the methanol. Ammonium hydroxide was added to pH 9 and the solution extracted repeatedly with chloroform. The chloroform solution was concentrated to about 50 cc., whereupon a colorless solid separated. The solid was filtered, identified as ammonium periodate, and rejected. The chloroform filtrate was evaporated to dryness, whereupon a colorless amorphous alkaloidal residue was obtained. The residue resisted all attempts at crystallization. The infrared spectrum of the material showed a broad (carbonyl) band in the region of 5.81–5.90 μ, but no absorption in the 5.00 to 5.80 μ region.

TABLE I.—CHROMIC ACID TITRATIONS

Alkaloid	Oxygen Equivalents Consumed, hr. 1	2	3
Cevadine orthoacetate acetate (V)	0.83	0.93	0.93
Cevadine orthoacetate (VI)	0.85	0.95	0.95

Chromic Acid Titrations.—The method described in Part XXXVIII (22) was used. Cevadine D-orthoacetate 4-acetate (V) ("monoacetyl-anhydrocevadin"), m. p. 280–281° (decompn.), $[\alpha]_D^{23}$ + 72° (c 0.95, chf.), and cevadine D-orthoacetate (VI) ("anhydrocevadin"), m. p. 214–215° (decompn.), $[\alpha]_D^{23}$ +97° (c 0.98, ethanol), were prepared according to the procedures of Stoll and Seebeck (20). The results obtained on chromic acid titration of the latter compounds are summarized in Table I.

REFERENCES

(1) Merck, G., *Ann.*, **95,** 200(1855).
(2) Schmidt, E., and Koppen, R., *ibid.*, **185,** 224(1877).
(3) Wright, C. R., and Luff, A. P., *J. Chem. Soc.*, **32,** 338 (1878).
(4) Bosetti, E., *Arch. Pharm.*, **221,** 82(1883).
(5) Ahrens, F. B., *Ber.*, **23,** 2700(1890).
(6) Freund, M., and Schwarz, H., *ibid.*, **32,** 800(1899).
(7) Horst, P., *Chem. Ztg.*, **26,** 334(1902).
(8) Stoll, A., and Seebeck, E., *Helv. Chim. Acta*, **35,** 1270 (1952).
(9) Pelletier, S. W., and Jacobs, W. A., *J. Am. Chem. Soc.*, **75,** 3248(1953).
(10) Kupchan, S. M., Lavie, D., Deliwala, C. V., and Andoh, B. Y. A., *ibid.*, **75,** 5519(1953).
(11) Fieser, L. F., and Fieser, M., "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 888.
(12) Kupchan, S. M., and Afonso, A., *J. Org. Chem.*, in press.
(13) Kupchan, S. M., and Afonso, A., THIS JOURNAL, **48,** 731(1959).
(14) Kupchan, S. M., and Ayres, C. I., *ibid.*, **48,** 735 (1959).
(15) Kupchan, S. M., *J. Am. Chem. Soc.*, **81,** 1921(1959).
(16) Kupchan, S. M., *ibid.*, **81,** 1925(1959).
(17) Kupchan, S. M., and Ayres, C. I., THIS JOURNAL, **48,** 440(1959).
(18) Kupchan, S. M., and Ayres, C. I., *J. Am. Chem. Soc.*, in press.
(19) Kupchan, S. M., Ayres, C. I., and Hensler, R. H., *ibid.*, in press.
(20) Stoll, A., and Seebeck, E., *Helv. Chim. Acta*, **35,** 1942 (1952).
(21) Levine, J. and Fischbach, H., THIS JOURNAL, **44,** 543 (1955).
(22) Kupchan, S. M., Ayres, C. I., Neeman, M., Hensler, R. H., Masamune, T., and Rajagopalan, S., *J. Am. Chem. Soc.*, in press.

Some Pharmaceutical Properties of Novobiocin*

By JOHN D. MULLINS and THOMAS J. MACEK

Novobiocin (Cathomycin) is an antibiotic produced in nutrient media by *Streptomyces spheroides*. Physical characteristics of the amorphous acid form of novobiocin were studied. The amorphous acid is biologically active, but proved metastable in aqueous suspension, reverting on standing to a less soluble, inactive crystalline form. Several distinct steps were identified during the process of reversion; the rate of reversion was found to be temperature dependent. Methods for suppressing crystallization of the amorphous acid are discussed. The properties of amorphous calcium novobiocin in formulation of aqueous suspensions were studied.

NOVOBIOCIN is an antibiotic substance produced in nutrient media by *Streptomyces spheroides*. Several authors have described its chemical, microbiological, and pharmacologic properties (1–7).

The antibiotic normally is produced as the crystalline monosodium salt. This form is absorbed following oral or parenteral administration and is effective against strains of Streptococcus, Staphylococcus, Proteus, Diplococcus, and Pasturella. Staphylococci seem to be particularly sensitive, as are several strains of penicillin-resistant Streptococci.

Novobiocin usually occurs as a white to cream-colored amorphous solid or a pale yellow crystalline solid, melting at 153 to 158°, with decomposition. The antibiotic is a weak dibasic acid having pK values of 3.8 and 9.4. It is soluble in methanol, ethanol, butanol, acetic acid, and dioxane; it is insoluble, or only slightly soluble, in ether, benzene, carbon tetrachloride, and chloroform. Although novobiocin acid is insoluble in water, both the mono and disodium salts are very soluble, but the rate of solution is quite slow.

Solutions of monosodium novobiocin are slightly alkaline and are incompatible with acids and acid salts. The antibiotic forms essentially insoluble salts with heavy metal ions. Insoluble salts are also formed by reaction with several amines such as procaine, phenylethylamine, morpholine, and N,N^1-dibenzylethylenediamine. Novobiocin also forms insoluble salts with basic antibiotics such as streptomycin, neomycin, and kanamycin. Several of these have been prepared and characterized (8).

In 0.1 *N* sodium hydroxide solution novobiocin exhibits an absorbance maximum at 307 mμ with $E(1\%, 1\text{ cm.}) = 600$. In phosphate buffer at pH 7.0 the absorbance maximum occurs at 305 mμ with $E(1\%, 1\text{ cm.}) = 343$. In acidic methanol (0.1 *N* hydrochloric acid in 90% methanol) the absorbance maximum occurs at 305 mμ with $E(1\%, 1\text{ cm.}) = 384$.

Novobiocin is optically active showing the following rotational characteristics:

$$[\alpha]_D^{25} = -44° \quad (C = 1\%, \text{ pyridine})$$

$$[\alpha]_D^{25} = -29° \quad (C = 1\%, 1\ N \text{ sodium hydroxide})$$

Titration curves for monosodium novobiocin with citric acid and with hydrochloric acid are shown in Fig. 1.

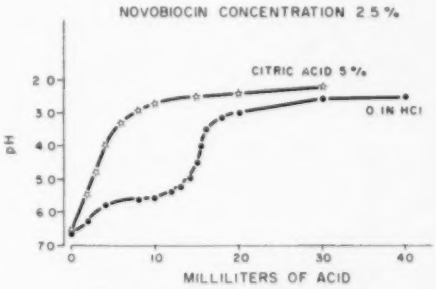


Fig. 1.—Novobiocin titration.

EXPERIMENTAL

Crystalline and Amorphous Novobiocin.—When an aqueous solution of the sodium salt is acidified novobiocin acid is usually obtained as an amorphous precipitate. However, the amorphous solid is metastable and is readily converted to a more stable crystalline form. The rate of conversion is temperature dependent and proceeds quite rapidly at higher temperatures.

Apart from the more obvious physical differences between crystalline and amorphous forms, an important though unexpected difference has been observed in biological availability. The crystalline acid is poorly absorbed and does not produce therapeutically adequate systemic levels of novobiocin following oral administration. The amorphous acid, on the other hand, is readily absorbed and is ther-

* Received August 21, 1959, from Merck Sharp and Dohme Research Laboratories, West Point, Pa.

The authors gratefully acknowledge the cooperation of Dr. John Baer and Mrs. Helen Skeggs in performing the biological tests, Dr. D. E. Williams for the X-ray measurements, and Mr. R. McGaughran for the infrared measurements. All these individuals are members of the staff of the Merck Sharp and Dohme Research Laboratories.

Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.

apeutically active. This difference in availability was thought to be related to differences in solubility in water and aqueous systems. Experiments to test the hypothesis were performed by shaking an excess of crystalline or amorphous novobiocin acid, both essentially less than 10 μ in particle size, with 400 ml. of 0.1 *N* hydrochloric acid at 25°. At hourly intervals samples were taken; ultraviolet absorbance was measured after filtration. When it became available, the same experiment was performed with amorphous calcium novobiocin. This salt was later found to be biologically active although not particularly soluble.

The data in Fig. 2, corrected for volume changes, indicate that both amorphous novobiocin acid and the amorphous calcium salt exhibited greater absorbance as a function of time than did the crystalline solid. This was interpreted to mean that the amorphous solids were at least tenfold more soluble in 0.1 *N* hydrochloric acid than was the crystalline solid. This difference in solubility might be expected to favor the absorption of the amorphous solid from the gastrointestinal tract.

Data showing differences in novobiocin plasma levels in dogs following oral administration of 12.5 mg./Kg. of each of amorphous novobiocin, amorphous calcium novobiocin, and crystalline novobiocin are shown in Table I.

Blood levels obtained with the sodium salt as a control, are also shown. Satisfactory absorption of the monosodium salt suggests that the free acid formed when this salt enters the stomach is either amorphous or of some other physical form suitable for absorption.

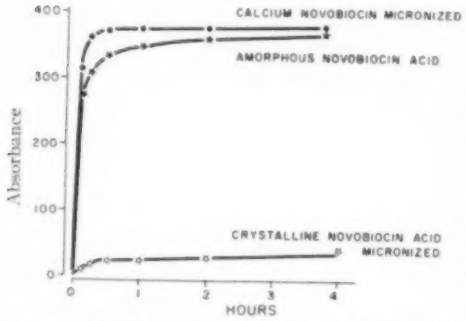


Fig. 2.—Absorbance of novobiocin in 0.1 *N* HCl at 305 mμ.

TABLE I.—NOVOBIOCIN PLASMA LEVELS IN DOGS FOLLOWING ORAL ADMINISTRATION OF DIFFERENT SOLID FORMS[a]

Hours after Dose	Sodium Novobiocin, mcg./ml. Plasma	Amorphous Novobiocin (Acid), mcg./ml. Plasma	Calcium Novobiocin, mcg./ml. Plasma	Crystalline Novobiocin (Acid)
1/2	0.5	5.0	9.0	[b]
1	0.5	40.6	16.4	[b]
2	14.6	29.5	26.8	[b]
3	22.2	22.3	19.0	[b]
4	16.9	23.7	15.7	[b]
5	10.4	20.2	13.8	[b]
6	6.4	17.5	10.0	[b]

[a] Dose = 12.5 mg./Kg.
[b] Not detectable

Crystallization of Suspensions.—Instability of amorphous novobiocin can be illustrated best by a description of the behavior of an aqueous suspension. When freshly prepared by acidification of a solution of the sodium salt, a suspension of amorphous novobiocin is white in color, the solid is gel-like and well suspended. The particles are approximately 1 to 5 μ in size. On standing, however, the suspension gradually becomes more dense, particle size increases, and there is greater settling of the suspended phase.

Microscopic examination of the suspension during the course of these changes reveals several other intermediate steps in the transformation process. A change in the amorphous particles is first revealed by the appearance of spheroid particles. These appear to be formed by the coalescence of a number of minute amorphous particles which become fixed together. The clustered particles tend to become more dense and show some increase in color; they assume a roughly spherical shape, and continue to increase in size in a roughly symmetrical manner. In larger spheroids concentric growth rings sometimes become visible. The spheroid particles attain a maximum diameter of approximately 30 μ. At this point by gross examination, the suspension is definitely more yellow in color and sedimentation rate has increased.

The second or crystallization step first becomes visible microscopically by the appearance of needle or rod-like crystals vertical to the periphery of the spheroids. Crystal growth then proceeds inward toward the center of the spheroid. Fragility of the spheroids increases with depth of crystallization until soon the novobiocin predominantly is present in suspension as intact or fragmented rosettes of needle-like crystals. The photomicrographs in Fig. 3 illustrate the various steps in the crystallization process.

The process of crystallization of novobiocin also has been examined by X-ray diffraction studies using a Norelco X-ray diffractometer. Figure 4 illustrates the intensity of X-ray diffraction from a crystalline sample as contrasted with little or no diffraction by a sample of amorphous novobiocin. It is interesting to note that spheroids are no longer amorphous by X-ray diffraction nor are they fully crystalline.

Examination of the infrared spectra of both the crystalline and amorphous forms of novobiocin revealed only minor differences and the spectra for the two forms were practically indistinguishable. The spectrum for the crystalline solid showed some evidence of increase in bond strength at several points.

The process of crystallization in suspensions was observed to be irreversible, as expected. On the other hand, rate of crystallization appeared to vary considerably with temperature. Indeed at room temperature crystallization could not be induced by vigorous shaking and scratching even with the addition of crystalline seed. Experiments, therefore, were conducted to determine the length of time required for spontaneous crystallization to take place in aqueous suspensions containing a therapeutically-useful concentration of amorphous novobiocin.

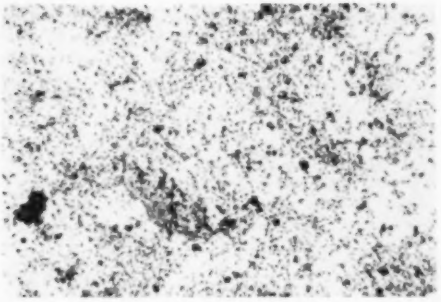


Fig. 3A.—Amorphous novobiocin (×344).

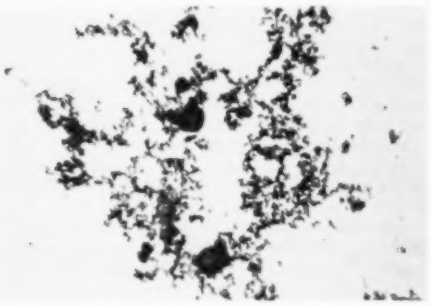


Fig. 3B.—Beginning spheroid formation (×344).

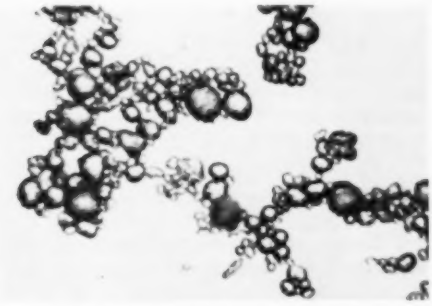


Fig. 3C.—Spheroid formation (×344).

Fig. 3D.—Spheroids with peripheral crystallization (×344).

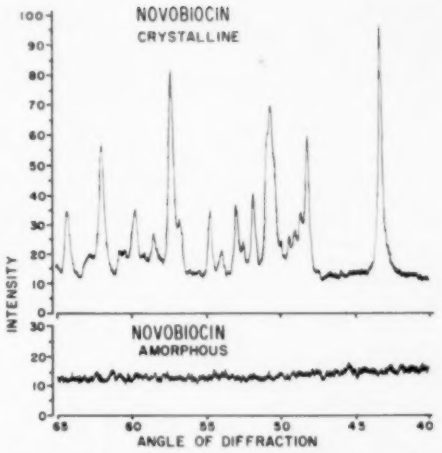


Fig. 4.—X-ray diffraction; amorphous and crystalline novobiocin.

A solution of sodium novobiocin, equivalent to 2.5% novobiocin acid, was adjusted to pH 3.5 with hydrochloric acid. Samples of the suspension of amorphous novobiocin acid which was formed were subdivided into glass bottles for storage at several temperatures. The specimens of stored suspensions were examined microscopically for crystallization at frequent intervals during storage. The end point was taken as the presence of both spheroids and crystals on the same microscopic field. Crystallization was observed to occur within a few hours at 60°, but only after six months at 25°. The data are shown in Table II.

TABLE II.—APPROXIMATE TIME REQUIRED FOR CRYSTALLIZATION OF NOVOBIOCIN SUSPENSION

Storage Temperature, °C.	Crystallization Time
60	4–6 hours
50	24 hours
37	22 days
25	6 months

As a continuation of these studies it was observed that suspensions containing 1.25% amorphous novobiocin required a longer time for crystallization. At a concentration of 0.625% amorphous novobiocin the time required for crystallization was approximately twice that found for the 2.5% samples.

Stabilization of Suspensions.—Even though aqueous suspensions containing 2.5% or more of amorphous novobiocin were found physically stable for six months at room temperature, these represented thermodynamically unstable systems. Crystallization of such suspensions in a shorter period of time or under more adverse conditions of storage and shipment could not be dismissed. On the other hand, crystalline acid was inactive and sodium novobiocin, although useful orally, could not be dispensed in ready-made liquid form because of chemical instability.

Inasmuch as the crystallization of suspensions of amorphous novobiocin was observed to be a stepwise process, it seemed reasonable that crystallization could be controlled further with additives. Such procedures certainly are well-known in the control of many crystallization operations.

Accordingly, a search was conducted for additives that would significantly retard or even prevent crystallization of aqueous suspensions of amorphous novobiocin. Suspensions of the amorphous acid at 2.5% concentration were prepared by adjusting aqueous solutions of sodium novobiocin to pH 3.5 with hydrochloric acid. Additives to retard crystal formation were generally present during acidification. In a few instances they had to be added in aqueous solution immediately afterward. The temperature and rate of addition of hydrochloric acid, rate of stirring, and volume of sample were kept uniform. All samples, together with appropriate controls containing no additives, were stored in glass bottles at 50°. These were examined microscopically at frequent intervals for signs of crystallization or spheroid formation. A list of additives tested and their effect on crystallization time is given in Table III.

TABLE III.—EFFECT OF ADDITIVES ON RATE OF CRYSTALLIZATION OF AMORPHOUS NOVOBIOCIN ACID IN AQUEOUS SUSPENSION

Additive	Rate of Crystallization
Benzalkonium chloride	Increased
Tween series[a]	Increased
Pluronic F-68[b]	Increased
Polyethylene glycol esters	Increased
Sucrose esters	Increased
Polyethylene glycol series	Increased
Lecithin	Increased
Dextran	Decreased slightly
Gelatin	Decreased slightly
Carboxymethylcellulose	Decreased slightly
Starch	Decreased slightly
Hydroxyethylcellulose[c]	Decreased slightly
Cyanoethylated starch	Decreased slightly
Acacia	Decreased slightly
Tragacanth	Decreased slightly
Casein	Decreased slightly
Lactalbumen	Decreased slightly
Bentonite	Decreased slightly
Methylcellulose	Decreased markedly
Polyvinylpyrrolidone	Decreased markedly
Cellulose acetate hydrogen phthalate	Decreased markedly
Sodium alginate	Decreased markedly
Propylene glycol algin[d]	Decreased markedly

[a] Atlas Powder Co.
[b] Wyandotte Chemicals Corp.
[c] Cellosize Carbide and Carbon Chemical Co.
[d] Kelcolloid Kelco Co.

The data show the surface-active agents as a class, regardless of ionic character, generally increased the rate at which crystallization took place.

A number of water-soluble or water-dispersible gums and substances generally regarded as protective colloids were found ineffective in retarding this crystallization process. Only few agents provided adequate protection against crystallization for really significant periods of time. Further, time of crystallization was found dependent upon concentration of the additive, as is shown in Table IV with

TABLE IV.—EFFECT OF METHYLCELLULOSE CONCENTRATION ON NOVOBIOCIN CRYSTALLIZATION RATE

Concentraton of Methylcellulose 1,500 c.p.s. as % of Total Volume	Time Required for Crystallization, days 50°	37°
0	1	22
0.1	2	25
0.25	3	35
0.50	5	60
0.75	7	About 90
1.00	25	>365

various concentrations of methylcellulose. The best agents for stabilizing suspensions of amorphous novobiocin against crystallization were methylcellulose, several types of alginic acid derivatives, such as sodium alginate or propylene glycol algin, and polyvinylpyrrolidone.

Suspension of Calcium Novobiocin.—The preparation of the calcium salt of novobiocin solved numerous problems previously encountered with the acid.

Calcium novobiocin proved to be a tasteless salt that was fully biologically active and perfectly stable in aqueous suspension though amorphous. Data showing solubility in dilute acid and availability in dogs were given previously.

Even though crystalline calcium novobiocin has been obtained from organic solvents, the amorphous salt is obtained in water by precipitation of sodium novobiocin with calcium chloride. Aqueous suspensions of the amorphous calcium salt have failed to crystallize in our laboratory studies even after twenty-four months of storage at room temperature and at 37°. On this basis, the addition of substances to retard crystallization has not been found necessary.

SUMMARY

1. The pharmaceutical properties together with some of the physical and chemical properties of novobiocin have been described.

2. The characteristics of the metastable amorphous form of novobiocin have been discussed. Different additives for stabilizing this biologically-active, metastable form are described.

REFERENCES

(1) Kaczka, E. A., Wolf, F. J., Rathe, F. P., and Folkers, K., *J. Am. Chem. Soc.*, **77**, 6404(1955).
(2) Hoeksema, H., Johnson, J. L., and Hinman, J. W., *ibid.*, **77**, 6710(1955).
(3) Wallick, H., Harris, D. A., Reagan, M. A., Ruger, M., and Woodruff, H. B., "Antibiotics Annual 1955–56," Medical Encyclopedia, Inc., New York, N. Y., 1956, p. 909.
(4) Verwey, W. F., Miller, A. K., and West, M. K., *ibid.*, p. 924.
(5) Smith, C. G., Dietz, A., Sokolski, W. T., and Savage, G. M., *Antibiotics & Chemotherapy*, **6**, 135(1956).
(6) Wilkins, J. R., Lewis, C., and Barbiers, A. R., *ibid.*, **6**, 149(1956).
(7) Taylor, R. M., Miller, W. L., and VanderBrook, M. J., *ibid.*, **6**, 162(1956).
(8) Chaiet, L., and Wolf, F., *ibid.*, **7**, 231(1957).

The Effect of Thixotropy on Suspension Stability*

By ERNEST C. FOERNZLER, ALFRED N. MARTIN, and GILBERT S. BANKER

This study was undertaken to investigate the effect of thixotropy on physical suspension stability. Two thixotropic suspending agents, bentonite and attapulgite, were evaluated in systems of varying concentrations for thixotropy, plastic viscosity, and yield value by employing a modified Stormer viscometer. A technique involving centrifugation was developed for the evaluation of stability. In the case of both suspending agents employed, the correlation of physical suspension stability and the degree of thixotropy of the suspending agent indicated that sedimentation velocity was directly proportional to the reciprocal of thixotropic area.

DUE TO THE NATURE of pharmaceutical suspensions and the fact that they are to be used as dosage forms, physical stability is of utmost importance. In attempting to make calculations concerning the physical stability of a pharmaceutical suspension, however, it is usually found that an extremely complex system must be dealt with, the parameters of which are in many cases quite difficult to measure and often impossible to express mathematically. Furthermore, the rates of involved physical processes are equally difficult to evaluate (1). Here it is earnestly felt that time and effort may be conserved if the experimentation is directed toward rheological methods.

The colloidal phenomenon of thixotropy is an important physical property of a variety of pharmaceutically acceptable suspending agents. Through utilization of thixotropy in the preparation of liquid suspensions it is possible to minimize or eliminate sedimentation and coagulation of the insoluble constituents which the product contains. Thus, a knowledge of the flow properties of thixotropic suspending agents should provide an insight into the formulation and development of suspended products of superior physical stability. Many pharmaceutical suspending agents are known to possess thixotropic flow properties. Among these are bentonite (2), hectorite (3), Veegum[1] (4), attapulgite (5), sodium carboxymethylcellulose (6), and Carbopol 934[2] (7). It is unfortunate that there exists at the present time no generally accepted method or specific conditions under which rheological analyses for thixotropic flow should be made. However, numerous publications have appeared in which the investigators have taken advantage of rheological evaluation procedures for analysis of thixotropic pharmaceutical systems (2, 6, 8–10). Although there will undoubtedly be some disagreement among workers representing methods of analysis which are not standardized, the information obtained from proper rheological evaluation of systems of this type is very useful for providing data by which the systems may be analyzed and controlled.

This study (*a*) evaluates the flow properties of two thixotropic suspending agents suitable for use in pharmaceutical systems, (*b*) evaluates the physical stability of medicinal products prepared with these suspending agents, and (*c*) attempts to obtain a mathematical relationship which would correlate thixotropy and physical suspension stability.

The two suspending agents employed in this project were bentonite, U. S. P.,[3] and Pharmasorb Colloidal[4] (activated attapulgite).

EXPERIMENTAL

Preparation of Suspension Bases.—Before preparing the suspension bases, both suspending agents were analyzed for moisture content by a standard Karl Fischer titrimetric method with an electrometric end point determination. Analysis of three samples for each suspending agent indicated moisture contents of 6.59 and 16.2%, respectively, for bentonite and Pharmasorb. Following moisture correction, bentonite suspension bases were prepared in concentrations of 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, and 7.0% (w/v) by adding the appropriate quantity of clay slowly in divided portions to 800–900 ml. of hot distilled water without stirring (U. S. P. method). The product was then allowed to hydrate at room temperature (23 to 27°) for twenty-four hours. At the end of the twenty-four hour hydration period the product was mixed at 3,600 ± 400 r. p. m. with a Waring Blendor[5] of 1 L. capacity for ten minutes. The volume of the product was then increased to 1,000 ml. and the contents mixed at the previous speed for an additional five minutes.

In the case of Pharmasorb Colloidal, the manufacturer recommends that the most stable suspension

* Received August 21, 1959, from the School of Pharmacy, Purdue University, West Lafayette, Ind.
Abstracted from a thesis submitted to the faculty of Purdue University in partial fulfillment of the requirements for the degree of Master of Science.
Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.

[1] R. T. Vanderbilt Co., New York, N. Y.
[2] B. F. Goodrich Co., Cleveland, Ohio.
[3] Fisher Scientific Co., Pittsburgh, Pa.
[4] Minerals and Chemicals Corp. of America, Menlo Park, N. J.
[5] Waring Products Corp., New York, N. Y.

bases be prepared by presoaking and/or pregelling the clay in water at 25° for a period of twelve to eighteen hours followed by stirring the mixture and diluting the pregelled concentrate (5). The Pharmasorb bases were therefore prepared at concentrations of 5.0, 6.0, 7.0, 8.0, and 9.0% (w/v) by adding to the appropriate quantity of clay a sufficient quantity of distilled water (25°) so that it just completely moistened the clay. The moistened clay was then allowed to soak for twenty-four hours at room temperature. Upon completion of the presoaking period, 800 ml. of distilled water was added, the product was mixed at 3,600 ± 400 r. p. m. for ten minutes with the Waring Blendor, the volume was increased to 1,000 ml., and the contents mixed for an additional five minutes as for the bentonite bases.

After preparation, all suspension bases were stored at room temperature in tightly closed, amber glass containers and allowed to hydrate further and age for at least fourteen days before rheological or stability measurements were begun. No preservative was employed in the bases since those acceptable for use were found to produce foaming during preparation of the bases, thereby creating undesirable conditions for rheological evaluation. The absence of a preservative did not affect the results over the period of experimentation. Although the pH of the bases was determined after preparation and at thirty-day intervals during the time the bases were employed, pH itself was not considered a variable in this study.

Rheological Evaluation.—A Stormer viscometer, properly modified for study of non-Newtonian flow, was employed for rheological evaluation in this project. Fischer has adequately described the modifications necessary in this respect (11). All rheological measurements were made at 25.0 ± 0.1° through application of a calibrated thermometer and a constant temperature bath. Before use, the viscometer was adequately calibrated with Newtonian standardization oils for use in the range of viscosity below one poise. Calculations used to obtain the instrumental constants K, K_v, K_f, and K_s and the working equations necessary for computing plastic viscosity (U) and yield value (f) were identical to those of Fischer (11). The instrumental constant K_s was evaluated for each flow curve from the appropriate weight/r. p. m. ratio for correction of the end-effect and consideration of the effective bob length (12).

After preliminary investigation of several procedures for quantitative determination of thixotropy, it was decided that the procedure most applicable to the manually operated Stormer viscometer was the production of a single hysteresis loop with accurate control of (*a*) the maximum rate of shear and (*b*) the time intervals between successive changes of weights. The maximum rate of shear was chosen at 400 r. p. m. (381.2 sec.$^{-1}$), while the time intervals between points on the curves were constant sixty-second periods. Flow curves were accepted for comparison only if a top rate of shear of 400 ± 5 r. p. m. was obtained. Although the time for production of the upcurve was not held exactly constant for each flow curve, it was felt that since all curves were made in exactly the same manner, this factor was at least approximately constant.

History effects (i. e., past treatments of the samples) were satisfactorily eliminated by allowing each sample to remain undisturbed in the viscometer cup and bob for two hours prior to beginning the flow curves. During this time it was necessary to prevent evaporation of moisture from the sample. This was accomplished by construction of a plastic, rubber lined top for the cup and bob. Following the experimental procedure, a plot of rate of shear *versus* shearing stress was made for each sample, and the parameters of plastic viscosity and yield value were calculated. The area of the hysteresis loop was then measured directly in square inches by application of a Dietzgen-Ott compensating planimeter,[6] and the resulting value was designated as the thixotropic area. Samples for each concentration of the suspending agents were run from four to ten times at two-to three-day intervals over a period of fourteen to twenty-one days, and mode values were taken to obtain the average rheological parameters.

Evaluation of Stability.—On standing, an initially uniform and relatively dense suspension of fine particles begins to settle at a constant rate with a well defined interface between the supernatant clear liquid and the settling particles. The rate at which the height of the interface changes with respect to time is known as the sedimentation velocity of the system. Preliminary work indicated that stability studies on the shelf at normal gravitational force would require periods ranging from six months to one year and more for evaluation of sedimentation velocities. Therefore, it was decided that accelerated studies should be employed. Multiplication of gravitational force was accomplished through application of a size 1, type SB International centrifuge.[7] The instrument was equipped with a hand brake, a rheostat speed control, a number 240 eight-place head, and a Waltham tachometer. Fifty-milliliter, Pyrex-glass graduated centrifuge tubes with short conical bottoms were used to contain the samples which were carried through radial distances of 10.0 and 20.1 cm. as measured from the shaft to the free surface and the tip of the sample respectively. Samples were prepared for centrifugation by incorporating 10% (w/v) zinc oxide (powder), U. S. P., in the suspension bases of varying concentrations of suspending agents. Incorporation was accomplished by sixty seconds of levigation with a small portion of the base and ten minutes of mixing with the remainder of the base by a laboratory power mixer. During centrifugation the centrifuge tubes were covered with polystyrene plastic sheeting to minimize evaporation and prevent aging effects. Each run in the centrifuge was made with four samples which were adequately balanced for minimum vibration, and care was taken when stopping the instrument to avoid convection currents.

Samples prepared with the bentonite bases were evaluated at 500, 750, 1,000 and 1,500 r. p. m., while those prepared with Pharmasorb were studied at 250, 500, 750, and 1,000 r. p. m. Following centrifugal sedimentation of the samples, graphs were constructed for evaluation of the initial free-fall sedimentation velocities by plotting the sediment volume *versus* time. In some cases it was

[6] E. Dietzgen Co., New York, N. Y.
[7] International Equipment Co., Boston, Mass.

necessary to employ the method of least squares for evaluation of the slopes of the sedimentation curves.

RESULTS AND DISCUSSION

Figures 1 and 2 show typical examples of the flow curves obtained in this study. Table I lists the average rheological values obtained for each suspension base and also indicates the sedimentation velocities obtained at 500 r. p. m. (a centrifugal force of approximately 1.85×10^6 dynes) after incorporation of 10% zinc oxide. The sedimentation rates obtained at 500 r. p. m. were used for correlation of stability and thixotropy since these values appeared to be the most consistent and since they were obtained for all but two (bentonite 6 and 7%) of the suspension bases evaluated. An attempt was made to extrapolate the sedimentation velocities to normal gravitational force by the use of semilog plots, but it was found that a sufficient number of points could not be obtained for valid extrapolation with the equipment employed.

By plotting the sedimentation velocities against the respective thixotropic areas it was found that hyperbolic functions appeared to exist for both suspending agents. Further investigation through plotting the sedimentation velocities *versus* the reciprocal of thixotropic area, Figs. 3 and 4, appeared to verify the hyperbolic relationships. These functions may be expressed in the form

$$V \cdot T = K \qquad \text{(Eq. 1)}$$

where V is the sedimentation velocity, T is the thixotropic area, and K is a material constant. Evaluation of the constant K resulted in values of 0.027 and 0.048 for bentonite and Pharmasorb, respectively. Thus it can be said that as a first approximation the sedimentation velocity is directly proportional to the reciprocal of the thixotropic area. It is felt that the source of variation from true linearity in the case of both bentonite and Pharmasorb was due largely to inaccurate determination of the sedimentation velocities.

The relationship obtained from Figs. 3 and 4 is of both theoretical and practical interest. Assuming that the sedimentation velocity of a particular suspension base containing some therapeutic ingredient could be evaluated under accelerated condi-

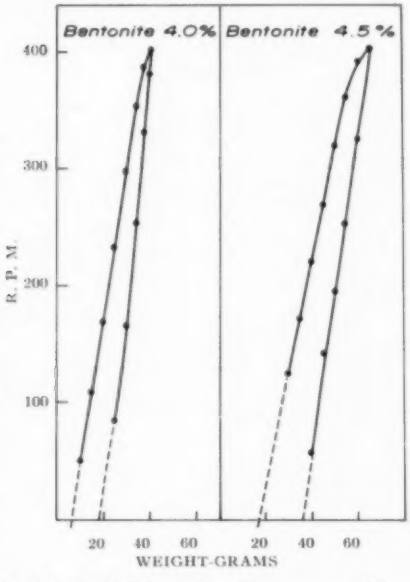


Fig. 1.—Typical bentonite flow curves. (Down curves on the left.)

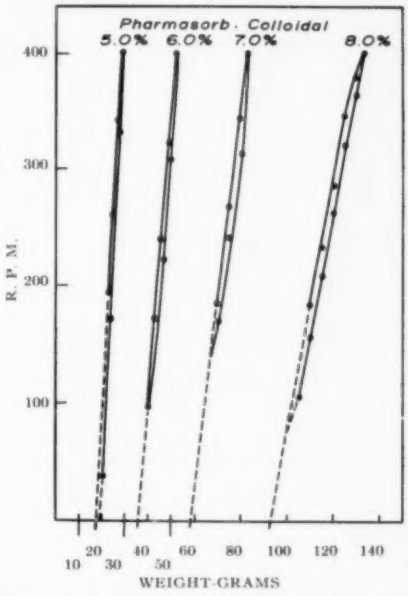


Fig. 2.—Typical Pharmasorb Colloidal flow curves. (Downcurves on the left.)

TABLE I.—AVERAGE RHEOLOGICAL PARAMETERS AND SEDIMENTATION VELOCITIES AT 500 R. P. M. IN ML./MIN.

Concn., % w/v	Thixotropic Area, Sq. In.	U Centipoise	f Dynes/cm.^{2}	Sedimentation Velocity
		Bentonite		
2.5	0.84	6.47	1.61	0.061
3.0	1.09	7.59	3.24	0.025
3.5	2.02	11.71	7.60	0.018
4.0	2.49	13.60	10.40	0.010
4.5	3.67	18.80	28.70	0.007
5.0	4.51	24.55	33.39	0.003
6.0	8.26	37.79	80.12	...
7.0	13.18	59.64	174.67	...
		Pharmasorb Colloidal		
5.0	0.06	4.23	32.15	0.800
6.0	0.14	6.37	59.79	0.400
7.0	0.24	10.76	101.79	0.200
8.0	0.49	16.92	175.64	0.100
9.0	0.84	22.67	245.25	0.050

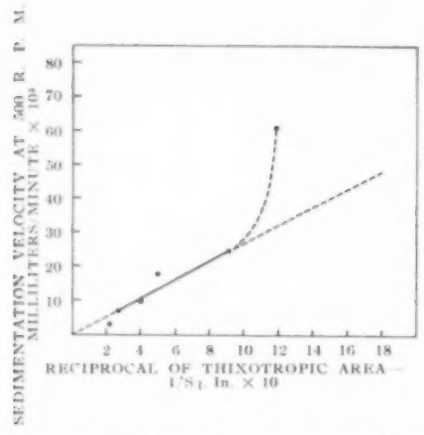


Fig. 3.—The stability-thixotropy relationship for bentonite.

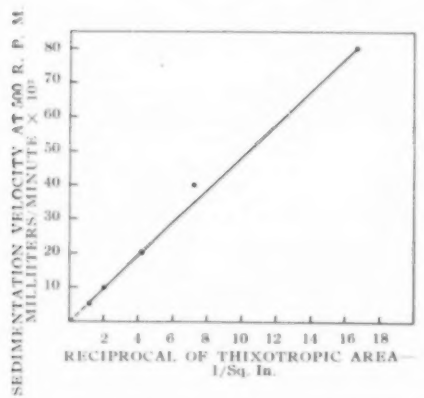


Fig. 4.—The stability-thixotropy relationship for Pharmasorb Colloidal.

tions and that the rate of sedimentation could satisfactorily be extrapolated to normal conditions, the degree of thixotropy required for optimum physical stability could be obtained. Conversely, it might be possible to predict the physical stability of a given suspension base after a rheological evaluation of thixotropy had been made. Of course, other properties such as plastic viscosity and yield value and the concentration of the system must also be taken into consideration before a more general correspondence between physical stability and rheological properties can be expected. Investigation of the effect of plastic viscosity and yield value on sedimentation velocity and of concentration on rheological properties is in progress, and an attempt to relate physical suspension stability mathematically to all three rheological properties has been planned for future discussion.

SUMMARY AND CONCLUSIONS

An attempt has been made to correlate thixotropy and physical suspension stability for two thixotropic pharmaceutical suspending agents; bentonite and Pharmasorb Colloidal.

A properly modified Stormer viscometer was used for rheological evaluation, and a technique involving centrifugation was employed for evaluation of stability under accelerated conditions.

An attempt to extrapolate sedimentation velocities obtained at accelerated rates to normal gravitational force did not appear to provide consistent results.

In the case of both suspending agents employed the correlation of physical suspension stability and the degree of thixotropy of the suspending agent indicated that the sedimentation velocity was directly proportional to the reciprocal of the thixotropic area, or that physical stability is directly proportional to thixotropy.

As a result of this work it may be concluded that both suspension evaluation and suspension formulation may be benefited by the application of proper rheological procedures.

Further investigation of physical suspension stability as a function of thixotropy, plastic viscosity, yield value, and concentration is currently in progress.

REFERENCES

(1) Higuchi, T., THIS JOURNAL, **47**, 657(1958).
(2) Bernstein, H. B., and Barr, M., *ibid.*, **44**, 375(1955).
(3) Macaloid Brochure, The Inerto Co., San Francisco, Calif., p. A-2.
(4) Veegum Technical Bulletin, No. 122, R. T. Vanderbilt Co., New York, N. Y.
(5) Pharmasorb Technical Bulletin, No. MDX-101, Minerals and Chemical Corp. of America, Menlo Park, N. J., 1958.
(6) deButts, E. H., Hudy, J. A., and Elliott, J. H., *Ind. Eng. Chem.*, **49**, 94(1957).
(7) Dittmar, C. A., *Drug and Cosmetic Ind.*, **81**, 446 (1957).
(8) Kostenbauder, H. B., and Martin, A. N., THIS JOURNAL, **43**, 401(1954).
(9) Bernstein, H. B., and Barr, M., *ibid.*, **46**, 41(1957).
(10) Ober, S. S., Vincent, H. C., Simon, D. E., and Frederick, K. J., *ibid.*, **47**, 667(1958).
(11) Fischer, E. K., "Colloidal Dispersions," John Wiley & Sons, New York, N. Y., 1950, pp. 147–190.
(12) Hamlow, E. E., "The Correlation of Several Rheological Methods for Measuring Newtonian and Non-Newtonian Materials," Ph.D. Thesis, Purdue University, West Lafayette, Ind., 1958.

Notes

A Note on the Photolytic Degradation of Anti-Inflammatory Steroids*

By W. E. HAMLIN, T. CHULSKI, R. H. JOHNSON, and J. G. WAGNER

PREVIOUSLY DESCRIBED (1–3) photolytic degradations of the A-ring of Δ^4-3-keto steroids and $\Delta^{1,4}$-3-keto steroids are of interest pharmaceutically since: (*a*) alcoholic solutions of such steroids are used in assay procedures and the steroid might degrade therein, and (*b*) ultraviolet light might deleteriously affect steroid-containing dosage forms.

Savard, *et al.* (1) showed that exposure of Δ^4-3-keto steroids to ultraviolet light caused saturation of the 4,5-double bond, or migration of the double bond, or both. Barton and Taylor (2) demonstrated that exposure of the $\Delta^{1,4}$-3-keto steroid, prednisone acetate, caused a complicated rearrangement in the A-ring of the steroid.

We have studied the effects of exposure of alcoholic solutions of hydrocortisone, prednisolone, and methylprednisolone to ordinary fluorescent laboratory lighting and of certain ophthalmic preparations, which contain steroid alcohols solubilized in aqueous solutions of Triton WR 1339,[1] to various sources of ultraviolet light. The commercial ophthalmic preparations studied were eye drops Optef 0.2%[2] which contains 0.2% hydrocortisone (Cortef[2]) and eye drops Neo-Deltef, 0.2%[2] which contains 0.2% prednisolone (Delta-Cortef[2]) and 0.5% neomycin sulfate. These sterile solutions are described in the patent of Johnson (4). The effects of exposure of these solutions, stored in different types of containers, to various sources of ultraviolet light were studied.

EXPERIMENTAL

Assay Methods.—The ultraviolet spectrophotometric method (5) and the isonicotinic acid hydrazide method (6) are specific for the Δ^4-3-keto and $\Delta^{1,4}$-3-keto groupings in the A-ring of the steroids. A significant decrease in potency as determined by these assay methods indicates degradation has occurred in the A-ring of the steroid. The 2,3,5-triphenyl tetrazolium chloride (tetrazolium) assay (7) involves reaction of the ketol side chain of the steroids. A significant decrease in potency as determined by this assay method indicates that degradation of the side chain at the 17-position has occurred. The paper chromatographic method (8) is specific for the intact steroid molecule being determined but has less precision than the other three methods.

Fade-Ometer Tests.—The solubilized steroid in aqueous solution was filled into vials and closed with the usual dropper fitments. Unexposed samples served as controls and provided the initial assay values. Some vials were wrapped in black paper and exposed to the ultraviolet light. These served as controls for the effect of heat. Unlabeled flint amber, or polyethylene vials were used as test specimens. A "wrap-around" commercial label was placed on other flint and polyethylene vials to test the protection afforded by this type of label. The various samples were exposed for six hours in a Type FDA-R Fade-Ometer manufactured by the Atlas Electric Devices Co., Inc., Chicago, Ill. Exposed and unexposed samples were assayed by the ultraviolet, tetrazolium, and paper chromatographic (papergram) methods.

Mercury Vapor Lamp Irradiation.—Samples of hydrocortisone in an aqueous solution of Triton WR1339 (eye drops Optef, 0.2%[2]) were packaged in flint and amber vials. A 100 watt, Type H4 G.E. mercury vapor lamp was mounted two inches from the samples to serve as a light source. After irradiation for specified times the samples were removed and assayed by the ultraviolet, tetrazolium, and papergram methods.

Miscellaneous Tests.—Samples of hydrocortisone in an aqueous solution of Triton WR1339 were exposed in unlabeled flint and amber vials to diffuse

TABLE I.—THE EFFECT OF ULTRAVIOLET LIGHT ON SOLUBILIZED HYDROCORTISONE IN EYE DROPS OPTEF, 0.2%, CONTAINED IN UNLABELED FLINT VIALS

Light Source	Duration of Exposure	Concentration of Steroid, mg./cc. Ultraviolet	Tetrazolium	Papergram
Fade-Ometer	0 hr.	2.07	1.98	2.18
	6 hr.	1.78	1.78, 1.93	1.54
Mercury vapor lamp	0 hr.	2.06	2.13	2.23
	6 hr.	1.91	1.88	2.04
	22 hr.	1.54	1.87	1.75
Diffuse light	3 days	2.23	2.12	2.10
	7 days	2.18	1.98	2.03
	14 days	2.01	2.00	2.00
Sunlight	1 day	2.12	2.02	1.89

TABLE II.—THE EFFECT OF ULTRAVIOLET LIGHT ON SOLUBILIZED PREDNISOLONE IN EYE DROPS NEO-DELTEF, 0.2%, CONTAINED IN UNLABELED POLYETHYLENE VIALS

Light Source	Duration of Exposure, hr.	Concentration of Steroid, mg./cc. Ultraviolet	Tetrazolium	Papergram
Fade-Ometer	0	2.17	2.14	1.67[a], 1.8[a], 2.01[b]
	6	0.586	2.25	0.20[a], 0.20[a], 0.24[b]

[a] Samples were stored for four months at room temperature before assay at two dilutions.

[b] Samples were stored for three months at 5°, then one month at room temperature before being assayed.

* Received October 23, 1959, from the Research Laboratories of The Upjohn Co., Kalamazoo, Mich.

[1] Registered trademark of Rohm and Haas Co.

[2] Registered trademarks of The Upjohn Co.

TABLE III.—RETARDATION OF PHOTOLYTIC DEGRADATIVE REACTIONS BY USE OF BLACK-WRAPPING, FLINT, OR POLYETHYLENE VIALS WITH WRAP-AROUND LABELS, AND AMBER VIALS

Steroid	Light Source	Duration of Exposure	Type of Packaging	Concentration of Steroid, mg./cc. Ultraviolet	Tetrazolium	Papergram
Hydrocortisone	Fade-Ometer	6 hr.	Black-wrapped flint	2.07	1.98	2.25
Hydrocortisone	Fade-Ometer	6 hr.	Unlabeled amber	2.06	1.97, 1.98	2.25
Hydrocortisone	Fade-Ometer	6 hr.	Labeled flint	2.10	2.07	...
Hydrocortisone	Fade-Ometer	6 hr.	Unlabeled amber	2.13	2.21	2.25
Hydrocortisone	Fade-Ometer	22 hr.	Unlabeled amber	2.29	2.13	2.27
Hydrocortisone	Diffuse light	3 days	Unlabeled amber	2.16	2.24	2.15
Hydrocortisone	Diffuse light	7 days	Unlabeled amber	2.13	2.16	2.14
Hydrocortisone	Diffuse light	14 days	Unlabeled amber	2.07	2.14	2.02
Hydrocortisone	Sunlight	1 day	Unlabeled amber	2.18	2.20	2.08
Prednisolone	Fade-Ometer	6 hr.	Black-wrapped polyethylene	2.17	2.14	1.73[a], 1.72,[a] 1.48[b]
Prednisolone	Fade-Ometer	6 hr.	Labeled polyethylene	1.73	2.14	0.91,[a] 0.98,[a] 1.13[b]

[a], [b] See footnotes to Table II.

light from a window (northern exposure) for three, seven, and fourteen days, and to sunlight for one day. In the latter case the exposure was approximately equivalent to six hours in direct sunlight. Inclement weather prevented controlled exposure for longer time intervals.

Solutions containing 40 mg./ml. of hydrocortisone, prednisolone, or methylprednisolone (Medrol®) in 95% ethanol were stored in 25-ml. volumetric flasks at room temperature (approximately 25°) under irradiation from ordinary fluorescent lighting[1] in the laboratory. Aliquots of the solutions were withdrawn after storage for specified times and assayed by the isonicotinic acid hydrazide and/or ultraviolet spectrophotometric methods.

RESULTS

Results of the exposure of solutions of hydrocortisone and prednisolone in aqueous solutions of Triton WR1339 to the various sources of ultraviolet light are given in Tables I, II, and III.

Results of the exposure of alcoholic solutions of hydrocortisone, prednisolone, and methylprednisolone to ordinary fluorescent laboratory lighting are given in Fig. 1. The slopes of the first order plots and the half-lives of the steroids under the conditions of the tests are given in Table IV.

DISCUSSION

Hydrocortisone in an aqueous solution of Triton WR1339 was found to undergo degradation when exposed to ultraviolet light in the Fade-Ometer only when the solution was packaged in unlabeled flint vials. A suitable "wrap-around" commercial label on the flint vial or an amber vial prevented degradation under the severe conditions of the test. The degradation which occurred in the unlabeled flint vial involved the A-ring as evidenced by the decrease in the U. V. assay. The results of exposing the aqueous hydrocortisone solution to the mercury vapor lamp confirmed this observation. Condition of exposure of the aqueous solutions of hydrocortisone to ultraviolet light in the Fade-Ometer and to the mercury vapor lamp were much more drastic than may be expected from exposure to diffuse light or even direct sunlight.

Prednisolone in aqueous solution of Triton WR1339 was photolytically degraded to a greater extent than hydrocortisone when exposed in the Fade-Ometer. The degradation of prednisolone, caused by the ultraviolet light, appeared to involve only the A-ring since the ketol side chain remained

[1] A laboratory approximately 16 × 22 feet was lighted by four fluorescent lights, each of which contained three 40-inch, 40-watt fluorescent tubes and which were mounted about 9 feet above the floor. Almost the entire northeast wall was glass. The samples were stored on a shelf two feet below and about two and one-half feet from one end of one of the fluorescent lights.

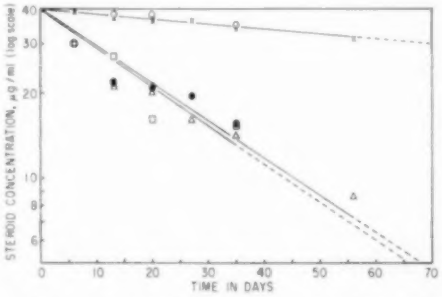


Fig. 1.—First order plots of the photolytic degradation of steroids when exposed to laboratory fluorescent lighting. Hydrocortisone: X, INH assay; O, U. V. assay. Prednisolone: Δ, INH assay; □, U. V. assay. Methylprednisolone: ●, INH assay.

TABLE IV.—SLOPES (k_1)[a] AND THEIR STANDARD DEVIATIONS (S_{k_1}) OF FIRST ORDER PLOTS, SHOWN IN FIG. 1, FOR PHOTOLYTIC DEGRADATION OF STEROIDS IN 95% ALCOHOL[b]

Steroid	$10^2\ k_1$	$10^4\ Sk_1$	$t^1/_2$[c] days
Hydrocortisone	0.188	0.944	160
Prednisolone	1.330[d]	8.304	22.6[e]
Methylprednisolone	1.386[d]	12.78	21.7[e]

[a] The slope or rate constant, k_1, is in units of $(2.303\ \text{days})^{-1}$.
[b] Solutions stored in 25-ml. volumetric flasks at room temperature (25°) under irradiation from the ordinary fluorescent laboratory lighting.
[c] The half-life of the steroid calculated by means of the expression $t^1/_2 = \log_{10} 2/k_1$.
[d] The slopes do not differ significantly.
[e] The half-lives do not differ significantly.

intact as indicated by the tetrazolium assays for the exposed samples compared with the controls.

The results obtained with the labeled polyethylene and flint vials in this study are valid only for the type of "wrap-around" commercial label utilized. If the label were smaller and did not cover as much of the surface area of the vial, equivalent results would not be expected. It is interesting from a pharmaceutical viewpoint that protection against photolytic degradation can be obtained by the use of a suitable label as well as by the use of amber glass. Additional protection against ultraviolet light is afforded by the cardboard carton which covers the vial except when the product is actually in use.

Ordinary fluorescent laboratory lighting causes photolytic degradation of hydrocortisone, prednisolone, and methylprednisolone as indicated by the results in Fig. 1. The slopes of the first order plots for the degradation of prednisolone and methylprednisolone were not significantly different but the slope for each of these steroids was 7 to 7.5 times the slope for hydrocortisone under the same test conditions. Hence, the two double bonds present in prednisolone and methylprednisolone make these steroids much more susceptible to ultraviolet light than the one double bond in the A-ring of hydrocortisone. Standard alcoholic solutions of these steroids should be stored in amber bottles or black-wrapped flint bottles at refrigerator temperature to ensure maintenance of the potency of the solutions.

REFERENCES

(1) Savard, K., Wotiz, H. W., Marcus, P., and Lemon, H. M., *J. Am. Chem. Soc.*, **75**, 6327(1953).
(2) Barton, D. H. R., and Taylor, W. C., *ibid.*, **80**, 244(1958).
(3) Belgian pat. 564,254 (1959).
(4) Johnson, R. H. (to The Upjohn Co.), U. S. pat. 2,880,138 (1959).
(5) Guttman, D. E., Hamlin, W. E., Shell, J. W., and Wagner, J. G., THIS JOURNAL, "Manuscript in preparation".
(6) Umberger, E. J., *Anal. Chem.*, **27**, 768(1955).
(7) Wagner, J. G., Dale, J. K., Schlagel, C. A., Meister, P. D., and Booth, R. E., THIS JOURNAL, **47**, 580(1958).
(8) Meister, P. D., Schlagel, C. A., Stafford, J. E., and Johnson, J. L., *ibid.*, **47**, 576(1958).

A Note on an Infrared Assay of Chloroform in Pharmaceutical Products*

By JOHN C. SOUDER and PATRICK DELUCA†

A quantitative infrared spectrophotometric assay for chloroform in pharmaceutical preparations has been developed. The procedure, which is sufficiently accurate for the control of filling operations, consists of extraction with carbon disulfide followed by estimation of chloroform by its characteristic infrared absorbance at 13.17 microns. Reproducibility of the method falls within ±2.9 per cent of the average.

THE USUAL PROCEDURES for determining chloroform are based upon hydrolysis and titration (2, 4, 6, 8) or the colorimetric reaction of Fujiwara (1, 3, 5, 7). These procedures are somewhat time consuming when used to control the filling of pharmaceutical products containing chloroform.

Chloroform is an ideal material for infrared spectrophotometric estimation. Chloroform is easily extracted from aqueous solutions into water-immiscible solvents such as carbon disulfide or cyclohexane which are suitable for use in infrared spectrophotometry. Carol (9) reported infrared determination of chloroform in drugs based on the 8.29 μ absorption maximum as the wavelength of choice using carbon disulfide as a solvent. In the present study the carbon-chlorine stretching band at 13.17 μ was used. The absorption peak at 13.17 μ was selected for quantitative estimation because it is unusually strong and it occurs in a relatively sparse region of the spectrum. Chloroform concentrations of 4 to 12 mg. per ml. of carbon disulfide were found to be useful in 0.1-mm. absorption cells. Carbon disulfide is quite transparent at 12 to 14 μ and thicker absorption cells could readily be used for lower chloroform concentrations.

* Received August 21, 1959, from Smith Kline and French Laboratories, Philadelphia 1, Pa.
† Present address. Temple University, School of Pharmacy, Philadelphia, Pa.
Presented to the Scientific Section, A. PH. A. Cincinnati meeting, August 1959.

EXPERIMENTAL

Preparation of Standards.—Standards were prepared by weighing known quantities of chloroform into 50-ml. volumetric flasks containing 40 to 45 ml. of ethyl alcohol. The flasks were then diluted to volume with ethyl alcohol. Volumetric aliquots of the alcohol-chloroform solution containing 100 to 300 mg. of chloroform were added to 75 ml. of distilled water in separatory funnels. The water-alcohol-chloroform standards were then extracted with 2–10 ml. and 1–5 ml. volumes of carbon disulfide, collecting the extracts in 25-ml. volumetric flasks. These carbon disulfide extracts are dry enough to use directly in infrared absorption cells without additional drying. A Perkin-Elmer model 21 infrared spectrophotometer equipped with sodium chloride optics was used for all the work reported here.

The absorption difference between 13.17 μ and 12.5 μ was used as an indication of chloroform content (Fig. 1).

Pharmaceutical samples were extracted and determined in the same way. Five different chloroform-containing liquid preparations were selected at random and extracted as described above for

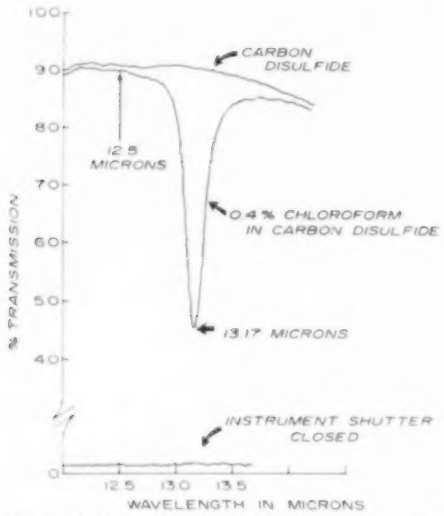


Fig. 1.—Infrared absorption of chloroform in carbon disulfide between 12 and 14 μ.

preparation of standards. In working with both standards and pharmaceutical preparations, it was necessary to force liquid into pipets with pressure rather than vacuum to avoid the loss of chloroform. Aliquots representing 100 to 250 mg. of chloroform were diluted 1:1 with water to make them more fluid and extracted with carbon disulfide. Only one of the five products had to be distilled before extraction to prevent emulsification. This one sample was diluted 1:1 with water and distilled through a water-cooled condenser into 25 ml. of alcohol cooled in an ice bath.

The same products were also assayed by alkaline hydrolysis and titration. Chloroform was hydrolyzed for titration by heating with 5 *N* alcoholic potassium hydroxide in pressure bottles for one hour in a boiling water bath. After hydrolysis, the solution was acidified with nitric acid and titrated potentiometrically with 0.1*N* silver nitrate solution. A comparison of results obtained by the two methods is shown in Table I. Three of the five samples required distillation to prevent interference when assayed by hydrolysis and titration.

TABLE I.—CHLOROFORM ASSAY RESULTS BY INFRARED AND HYDROLYSIS-TITRATION PROCEDURES

Product	Chloroform Found By Infrared Assay, mg./50 ml.	Chloroform Found By Alkaline Hydrolysis and Titration, mg./50 ml.
A	233.6	239.2
B	251.0	242.0
C	86.3	87.7
D	143.4	134.6
E	78.6	74.6

TABLE II.—REPRODUCIBILITY OF INFRARED CHLOROFORM ASSAY

Chloroform Assays in mg./50 ml.	
Run 1	Run 2
137.1	137.2
136.2	136.3
136.2	135.5
134.5	133.2

Average = 135.8 mg./50 ml.
Standard Deviation (σ) = 1.3 mg./50 ml.
3 × Standard deviation = 3.9 mg./50 ml. or ±2.9%.

Reproducibility of the infrared method was tested by running two groups of four aliquots from a single sample. The results obtained are shown in Table II. Standard deviation was found to be 1.3 mg./50 ml. or about 1%. For this same run of eight samples, the reproducibility (3 × S. D.) was ±2.9%.

REFERENCES

(1) Feldstein, M. and Klendshoj, N. C., *Can. J. Med. Technol.*, **17**, 127(1955).
(2) Stainier, C., and Grosjean, L., *Ind. Chim. Belge*, **20**, Spec. No., 551(1955).
(3) Sasaki, D., *Nisshin Igaku*, **42**, 344(1955).
(4) Kiba, T., and Terada, K., *J. Chem. Soc. Japan, Pure Chem. Sect.*, **75**, 196(1954).
(5) Kubalski, J., *Acta Polon. Pharm.*, **10**, 269(1953); **11**, 39(1954).
(6) Hanna, J. G., and Siggia, S., *Anal. Chem.*, **22**, 569 (1950).
(7) Burgen, A. S. V., *Brit. Med. J.*, **1**, 1238(1948).
(8) Gauzer, E., and Pashaeva, K., *Myasnaya Ind., S. S. S. R.*, **19**, 82(1948).
(9) Carol, J., *J. Assoc. Offic. Agr. Chemists*, **38**, 628(1955).

A Note on the Occurrence of 2,6-Dimethoxybenzoquinone in *Rauwolfia vomitoria**

By S. MORRIS KUPCHAN and MANG E. OBASI

RAUWOLFIA VOMITORIA Afz. originates in Africa in the region from upper Guinea and Uganda to Angola and Nyasaland (1). Roots of *R. vomitoria* have been shown to contain the important hypotensive and sedative alkaloids reserpine and rescinnamine, as well as several other indole alkaloids, and the roots of this Apocynaceous plant represent an important commercial source of reserpine (1).

The work reported herein concerns a nonalkaloidal constituent of an alkaloid-rich extract of *R. vomitoria*. The extraction procedure consisted of extracting, with chloroform the powdered *R. vomitoria* root, previously wetted with water. The chloroform extracts were evaporated to dryness and the residue was dissolved in methanol-acetic acid On standing, a brown solid separated in crystalline form (*ca* 0.002% based on root).[1]

Recrystallization of the crude brown crystalline solid from dioxane afforded light yellow needles, m. p. 254–255°. Analysis of the compound afforded results which supported a $C_8H_8O_4$ formula. Reduction with sodium bisulfite gave a dihydro derivative, $C_8H_{10}O_4$, which reverted to the original compound upon exposure at room temperature to air oxidation. Bromination in carbon disulfide afforded a dibromo derivative, $C_8H_6Br_2O_4$, and reductive acetylation gave a diacetate, $C_{12}H_{14}O_6$. The foregoing characteristics led to identification of the compound as 2,6-dimethoxybenzoquinone, and the identification was confirmed by direct comparison with an authentic synthetic sample prepared by oxidation of pyrogallol trimethyl ether (2, 3).

The isolation of 2,6-dimethoxybenzoquinone from *Adonis vernalis* of the family *Ranunculaceae* was reported some years ago (4). 2,6-Dimethoxybenzoquinone has been shown to exert strong bacteriostatic activity against a variety of microorganisms (5–8). One might speculate that the teleological significance of the occurrence of 2,6-dimethoxybenzoquinone in the plant kingdom may be associated with the bacteriostatic activity; perhaps this compound plays a role in the defense of plants against pathogens.

EXPERIMENTAL

Melting points are corrected for stem exposure. Infrared spectra were determined on a Baird model B double beam infrared recording spectrophotometer. Ultraviolet absorption spectra were determined on a model 11 MS Cary recording spectrophotometer and chloroform was used as solvent. Microanalyses were carried out by Drs. Weiler and Strauss, Oxford, England.

Purification of 2,6-Dimethoxybenzoquinone Derived from Rauwolfia vomitoria.—The brown crystalline solid obtained from methanol-acetic acid in the extraction procedure was recrystallized twice from dioxane with Norite treatment. Light yellow needles were obtained, m. p. 254–255°. The analytical sample was further purified by sublimation.

Anal.—Calcd. for $C_8H_8O_4$: C, 57.15; H, 4.80; OMe, 36.92. Found: C, 56.93; H, 4.70; OMe, 37.38.

Reduction of the Naturally-Derived 2,6-Dimethoxybenzoquinone.—A suspension of 2,6-dimethoxybenzoquinone (400 mg.) in water (5 cc.) was heated on the steam bath and treated dropwise with 20% sodium bisulfite solution (3.5 cc.). The yellow solid dissolved to form a colorless solution. Upon cooling, a colorless crystalline solid separated (250 mg.). Recrystallization from water afforded 2,6-dimethoxyhydroquinone in the form of colorless needles (200 mg.), m. p. 163–164°. [Reported m. p. 160° (4)].

Anal.—Calcd. for $C_8H_{10}O_4$: C, 56.45; H, 5.92; OMe, 36.47. Found: C, 56.20; H, 5.95; OMe, 38.45.

A solution of the hydroquinone in 95% alcohol was allowed to stand at room temperature overnight. A brown crystalline solid separated which afforded pure 2,6-dimethoxybenzoquinone, m. p. 254–255°, after recrystallization from dioxane.

Reductive Acetylation of the Naturally-Derived 2,6-Dimethoxybenzoquinone.—To a suspension of 2,6-dimethoxybenzoquinone (500 mg.) in acetic anhydride (2.5 cc.) was added zinc powder (1.0 Gm.) and powdered anhydrous sodium acetate (100 mg.). The mixture was heated gently until the yellow color disappeared, and then boiled for two minutes. The hot supernatant solution was decanted from the inorganic residue; the residue was washed with hot, glacial acetic acid (4 cc.) and the washings were added to the supernatant. The combined solution was heated, treated carefully with water to decompose excess acetic anhydride, and then further to turbidity. Upon cooling, 2,6-dimethoxyhydroquinone diacetate separated in the form of colorless crystals (525 mg.), m. p. 125–127°. Recrystallization from dilute alcohol gave colorless needles, m. p. 127–128°. [Reported m. p. 129–129.5° (9)].

Anal.—Calcd. for $C_{12}H_{14}O_6$: C, 56.70; H, 5.55. Found: C, 55.87; H, 5.24.

Bromination of the Naturally-Derived 2,6-Dimethoxybenzoquinone.—To a suspension of 2,6-dimethoxybenzoquinone (350 mg.) in carbon disulfide (5 cc.) was added excess bromine in carbon disulfide, and the solution was allowed to stand at room temperature overnight. Evaporation to dryness with a gentle current of air left a red crystalline residue. Recrystallization from absolute alcohol with Norite treatment gave 2,6-dimethoxy 3,5-dibromobenzoquinone in the form of red

* Received December 11, 1959, from the Department of Pharmaceutical Chemistry, University of Wisconsin, Madison.

Supported in part by a grant from the National Institutes of Health [H-2952 (C2)].

[1] The isolation and preliminary purification was done by Riker Laboratories, Inc., Northridge, Calif. We gratefully acknowledge the generosity of Mr. Murle W. Klohs in forwarding to us the crude crystalline material and information concerning its physical constants.

feathery plates (270 mg.), m. p. 177–178°. [Reported m. p. 175° (10)].

Anal.—Calcd. for $C_8H_6Br_2O_4$: C, 29.48; H, 1.86; Br, 49.03. Found: C, 30.06; H, 1.77; Br, 48.75.

Synthesis of 2,6-Dimethoxybenzoquinone.—2,6-Dimethoxybenzoquinone, m. p. 254–255°, was synthesized by oxidation of pyrogallol trimethyl ether with nitric acid in ethanol (2, 3). The melting point was not depressed upon admixture with the naturally-derived sample. Infrared and ultraviolet spectral comparison of the respective samples afforded positive proof of identity.

REFERENCES

(1) Woodson, R. E., Youngken, H. W., Schlittler, E., and Schneider, J. A., "Rauwolfia: Botany, Pharmacognosy, Chemistry, and Pharmacology," Little, Brown and Company, Boston, Mass., 1957.
(2) Graebe, von C., and Hess, H., *Ann.*, **340,** 232(1905).
(3) Baker, W., *J. Chem. Soc.*, **1941,** 665.
(4) Karrer, W., *Helv. Chim. Acta*, **13,** 1424(1930).
(5) Oxford, A. E., *Chem. & Ind.*, **61,** 189(1942).
(6) Wiedling, S., *Acta Pathol. Microbiol. Scand.*, **22,** 379(1945).
(7) Hoffmann-Ostenhof, O., and Fellner-Feldegg, H., *Monatsh.*, **80,** 720(1949).
(8) Rao, K. R., and Seshadri, T. R., *J. Sci. Ind. Research India*, **14C,** 189(1955).
(9) Huisman, H. O., *Rec. trav. chim.*, **69,** 1133(1950).
(10) Hunter, W. H., and Levine, A., *J. Am. Chem. Soc.*, **48,** 1608(1926).

Book Notices

British Pharmaceutical Codex 1959. Published by direction of the Council of the Pharmaceutical Society of Great Britain. The Pharmaceutical Press, 17, Bloomsbury Square, London, W. C., England, 1959. Available in the U. S. from Rittenhouse Book Store, 1706 Rittenhouse Square, Philadelphia 3, Pa. xxix + 1,301 pp. 14.5 x 22.5 cm. Price 70*s*.

This seventh edition of the British Pharmaceutical Codex" (first B. P. C. in 1907) carries on the aim of providing information on drugs in common use throughout the British Commonwealth. Included are many drugs, not official in the "British Pharmacopoeia," for which tests and standards have been developed.

Inclusion of many drugs that have been developed since the 1954 edition of the B. P. C. has been offset by deletions and revisions to the extent that B. P. C. 1959 has forty pages less than B. P. C. 1954. Monographs on official drugs indicate that the substance must comply with the requirements of the "British Pharmacopoeia." All monographs include statements on actions and uses, and give dosage forms along with a brief descriptive note on each preparation. Dosage forms that are not in the B. P. are given in detail in a "Formulary" section of the B. P. C. Thus, under calamine in the "General Monographs" section, there are given calamine lotion B. P. and four unofficial preparations of calamine. The latter four preparations are treated more fully in the B. P. C. "Formulary" section, giving the formulas, procedures for preparation, and standards.

The policy governing admissions of drugs into B. P. C. 1959 is the same as for B. P. C. 1954. This was discussed in the review of B. P. C. 1954 in THIS JOURNAL, **44,** 190 (1955). It is very similar to the admissions policy for our "National Formulary." Seventy new monographs have been added to the "General Monograph" section and 79 dosage forms (45 of which were in B. P. 1953 but not in B. P. 1958) have been added to the "Formulary" section. Deleted were 99 general monographs (including leech) and 120 formulary monographs.

Three new appendixes (XIV, XV, and XVI) deal with milliequivalents to assist in the preparation of parenteral infusion solutions, biological assays for chloramphenicol eye ointment and for neoarsphenamine, and limits on diameters of tablets.

The Codex Revision Committee has performed its task well in preparing this edition of a valuable reference book for pharmacy.

Organic Syntheses, Vol. 39. Edited by MAX TISHLER. John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, N. Y., 1959. 114 pp. 15 x 23 cm. Price $4.

This volume includes methods of preparation for: N-(*p*-acetylaminophenyl)rhodanine, benzeneboronic anhydride, 2,5-diamino-3,4-dicyanothiophene, di-*n*-butyldivinyltin, dicyanoketene ethylene acetal, 9,10-dihydroxystearic acid, N,N-dimethylcyclohexylmethylamine, *p*-dithiane, ethyl (1-phenylethylidene)cyanoacetate, indazole, indole-3-aldehyde, 2-mercapto-4-amino-5-carbethoxypyrimidine and 2-mercapto-4-hydroxy-5-cyanopyrimidine, methyl cyclopentanecarboxylate, methylenecyclohexane and N,N-dimethylhydroxylamine hydrochloride, 9-methylfluorene, 3-methyl-2-furoic acid and 3-methylfuran, methyl 3-methyl-2-furoate, β-methyl-β-phenyl-α,α'-dicyanoglutarimide, β-methyl-β-phenylglutaric acid, 1-phenyl-1-penten-4-yn-3-ol, phenylpropargyl aldehyde diethyl acetal, tetraacetylethane, tetracyanoethylene, *p*-tricyanovinyl-N,N-dimethylaniline, 2,4,4-trimethylcyclopentanone, α,α,β-triphenylpropionitrile, triptycene, and trithiocarbodiglycolic acid. A cumulative subject index for volumes 30–39 in this series is appended.

Steric Course of Microbiological Reactions. By Ciba Foundation Study Group No. 2. Little, Brown & Co., 34 Beacon St., Boston 6, Mass., 1959. vii + 115 pp. 12 x 18.5 cm.

A compilation of the papers and discussions at the symposium of the Ciba Foundation study group No. 2, March 17, 1959. References and an index are appended.

of operational improvement, cost reductions, labor, staff sales relations. Age 42. Interested production, plant, General Manager, $20,000 plus level. Box 6-5
TRIPLE THREAT—Plt. Eng.-Dev. Eng.-Sales Eng. Desire responsible position with small to medium size company who really needs an aggressive engineer with imagination and initiative. Not afraid of hard work or challenging problems. Prefer New York Area. B.S.Ch.E. 1950, five years Plant Engineer in heavy industry, Plastics Diploma, three years Development Engineer rubber industry. Presently employed. Box 5
PROJECT ENGINEER — B.Ch.E., M.S., P.E. Nine years' experience in process and project engineering of chemical and petrochemical plants with major company. Supervising engineer, group leader in [illegible] design engineering, pilot plant operations. Desire position as [illegible] manager [illegible] minimum salary [illegible]. Box 8-5
EMULSION STABILIZER
wanted for new pharmaceutical product. Must be compatible with unusual formula.
PRODUCTION MANAGEMENT ENGINEERING
[illegible] M.Ch.E. Fifteen [illegible] experience in the [illegible] and aerosol packaging plants. Plant—manager, process engineer, process evaluation and development, production supervision, seeking responsible position in production management. Present salary $11,000. Box 15-5
CHEMICAL ENGINEER—B.Ch.E. Has served as a technical advisor to an oil refinery since graduation, have also done instrumentation and automation studies. Desire position in process development or design. Age 22. Single and willing to travel. Box 16-5
CHEMICAL ENGINEER—B.S., graduate courses past M.S. Taught Chemical Engineering six
CHEMICAL ENGINEER — Ph.D., Seventeen years' experience in organic chemical plant process design, [illegible] studies, chemical [illegible] supervision, construction. New York Metropolitan Area. Box 7-5
SUSPENDING AGENT
wanted — must be capable of suspending high solids at low viscosities.
CHEMICAL ENGINEER—B.S. Ch. E. 1953. Five years experience in process development, product [illegible] exclusively engaged in the development of [illegible] now in the pilot plant status. Seeking responsible position in process development. Will consider only companies in vicinity of Boston, Mass. Box 5-6.
CHEMICAL ENGINEER—Rocket experience, [illegible] years R & D propellants, propulsion, [illegible] experience.—broad background design, analysis, thermodynamics, high energy systems, materials, processes. Publications, patents. M.Ch. E. Advanced graduate courses, M.E. 33. Desire challenging opportunity in propellants and rocketry. Eastern location. Box 6-6
CHEMICAL METALLURGICAL ENGINEER — M.S. Extensive specialized education in metallurgy, chemistry, chemical engineering. Experience in consummable electrode melting and metallurgy of reactive metals, molten salt reactions, corrosion, electrochemistry, high temperature reactions and thermodynamics, citizen, cleared, 34, single. Box 7-6.
CHEMICAL ENGINEER—B.S., veteran, age 38, family. Nine years' production, technological, and pilot plant experience in petrochemicals. Process evaluation. Catalysis. Resume available. Desire responsible technological or production position in Houston area. Box 8-6.
CHEMICAL ENGINEER — B.Ch.E. Age 26,
CHEMICAL ENGINEER—B.S., graduate credits. Seven years' varied experience in petroleum and chemical process design, project engineering, and construction. Proven technical and management ability. Desire responsible position in medium or small chemical company. Eastern or Midwestern U.S. Box 20-6.
PRODUCTION MANAGEMENT ENGINEERING — Licensed. Thirty years diversified experience in heavy chemicals, design, construction and operation. Have been chief engineer of two major chemical companies and plant manager. Seeking responsible position in production management. Box 21-6.
CHEMICAL ENGINEER—B.S. M.I.T. 1952. Four years' experience in pilot plant supervision, process development and research. Graduate work for M.B.A. Desire responsible position with progressive company. Married, two children. Box 22-6.
CHEMICAL ENGINEER — B.S.Ch.E. Age 37. Desire position as engineering supervisor or specialist. Experience in polymer field includes development, economics. Five years as [illegible] supervisor, [illegible] resin [illegible]. Present salary $10,000. [illegible]
THICKENER
wanted for medicated hand cream. Must be able to keep cream smooth and soft for full shelf life.
CHEMICAL ENGINEER — Production plant [illegible] plant design [illegible] of substantial cost [illegible] profit improvements. Self starter with a shirt sleeve approach when necessary for leadership. Publications. Family. Fifteen years' substantial experience in the petroleum and petro-chemical industry. Desire connection with a reasonable opportunity for advancement in either production or production supporting activity. Box 17-6.
PRODUCTION MANAGEMENT ENGINEERING

VEEGUM® CAN APPLY FOR ALL THREE JOBS

AS AN EMULSION STABILIZER VEEGUM excels. Less than 1% will increase stability of oil-in-water emulsions.

AS A SUSPENDING AGENT, VEEGUM gives more complete suspension and easier redispersion at lower viscosities than organic gums.

AS A THICKENER, aqueous dispersions of VEEGUM thicken slightly with heat. Therefore VEEGUM maintains formula consistency at higher than normal storage temperatures. Thixotropy gives it the suspending ability of a gel.

Yes, VEEGUM can handle all three jobs well. Wouldn't you like a sample for your laboratory? You will find VEEGUM the answer to difficult formulation problems.

USE THIS COUPON TO GET FAST ACTION

R. T. VANDERBILT CO., SPECIALTIES DEPARTMENT
230 PARK AVENUE, NEW YORK 17, N. Y.

( ) Please send VEEGUM Bulletin 53B ( ) Send sample of VEEGUM

State Application _____

Name _____

Title _____

(Please attach coupon to your Company letterhead) P - 7

Designed with good taste!

Planning pleasant palatability in new pharmaceuticals and proprietary medicinals is much simpler today due to the many achievements of modern flavor technology.

"SEALVA" dry flavors (hermetically-sealed inert flavoring materials of exceptional quality) offer special advantages for products in powder, grain, flake, tablet and oil-suspension form.

"ALVA" flavor constituents developed specifically for controlling unpleasant taste in liquid pharmaceuticals products.

The pharmaceutical section of the Alva Research Laboratory is unmatched in experience and ability in treating unusual palatability problems in medicinals. Each problem is treated individually. May we help you?

SEE US AT THE I F T MEETING IN SAN FRANCISCO BOOTHS 15-16

Alva FLAVORS

IFF

VAN AMERINGEN-HAEBLER • POLAK & SCHWARZ

INTERNATIONAL FLAVORS & FRAGRANCES INC.

417 Rosehill Place, Elizabeth 2, N. J.

Leading Creators and Manufacturers in the World of Flavor

ARGENTINA AUSTRIA BELGIUM BRAZIL CANADA ENGLAND FRANCE GERMANY HOLLAND INDONESIA ITALY NORWAY SOUTH AFRICA SWEDEN SWITZERLAND